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THE SIGNIFICANCE OF THE MONOCYTES IN AGRANULOCYTOSIS (LEUKOPENIC INFECTIOUS MONOCYTOSIS)*

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Considerable interest in the condition known as agranulocytosis has been aroused since 1922, at which time Schultz[30] published a report of his cases. Due to his precise description of the disease and its characteristic blood picture, its recognition and diagnosis have been made possible during life. It is remarkable that only two genuine cases of this condition appeared in the literature prior to 1922, namely, the case reported by Brown[4] in 1902, being the first one published in this country, and the case reported by Tuerk[32] in 1907. It seems likely that other cases occurred but had not been recognized or, in all probability, had been classified as severe septic conditions, especially malignant diphtheria. In this manner they escaped notice.

There has apparently been a marked increase in the incidence of agranulocytosis. Of special interest is the suggestion of Kracke[22] of a possible relationship between the widespread use of coal tar products and the marked increase in the number of cases of agranulocytosis. Not only has this belief been ascribed to by Kracke[23] himself, but also by Watkins,[34] Madison and Squier,[35] Hoffman[18] and Holten.[19] It has been known for some time that in a small percentage of patients receiving anti-luetic treatment, agranulocytosis, as well as aplastic anemia—has resulted from the toxic action of arsenobenzol. It is now recognized that a similar action may occur from amidopyrine and dinitrophenol. Agranulocytosis, however, may likewise develop without relation

* Read before the Fourteenth Annual Convention of the American Society of Clinical Pathologists, held at Atlantic City, New Jersey, June 7 to 9, 1935.

to drugs, as in the cases reported by Jackson,[20] Fitz-Hugh,[11] and others.

The condition shows a strong resemblance to various diseases including tonsillitis, laryngitis, "trench-mouth," diphtheria, grippe, pneumonia, aplastic anemia, leukemia, infectious mononucleosis or monocytosis, and sepsis. The differentiation from these diseases in the early stages is best accomplished by means of a blood examination which shows the characteristic profound leukopenia and neutropenia.

Observation on a large series of cases has convinced the writers that it is possible to differentiate three main types of agranulocytosis at the onset of symptoms, according to the predominance of certain cells, namely: (1) agranulocytosis with relative lymphocytosis; (2) agranulocytosis with unusual monocytosis (leukopenic infectious monocytosis), (Bix,[1] Rosenthal;[27] (3) hypo-leukocytic angina in which all the symptoms of agranulocytosis are present, including leukopenia, but without much change in the differential blood count (Rosenthal and Kugel).[28]

The object of the present communication is to report, in addition to the eight cases previously published,[27] fourteen cases of agranulocytosis with monocytosis (leukopenic infectious monocytosis) and also to show the possible relation of this form of agranulocytosis in certain cases to drugs.

CASE REPORTS

Leukopenic infectious monocytosis with recovery

No history of drugs

Case 1. Miss E. K., a buyer, fifty-three years of age, was observed by Dr. L. R. Tuchman, in the Private Pavilion of Mount Sinai Hospital, November, 1931. Past history, negative, except for frequent attacks of sore throat, the last having occurred seven months before admission. Present illness began suddenly, with chilly sensations and one shaking chill lasting about ten minutes. She gradually became worse, with malaise, marked weakness, fever and sore tongue. On November 10th, she lapsed into a stupor. The use of drugs was denied. When admitted to the hospital, the patient was toxic and stuporous, but could be aroused; a large, whitish, necrotic ulceration was observed at the tip of the tongue; a few petechiae were present on both thighs. The spleen was palpable just below the margin of the ribs.

Laboratory data: The following characteristic blood picture was present on admission:

Hemoglobin	70 per cent
Erythrocytes	4,450,000
Leukocytes	900
Platelets	280,000
Neutrophils:	
Non-segmented	1 per cent
Lymphocytes	69 per cent
Monocytes	23 per cent
Myeloblasts	3 per cent
Macrophages	4 per cent

A blood culture (performed prior to admission), revealed a heavy growth of hemolytic streptococci.

Course: The patient was apparently getting worse. Treatment with pentose-nucleotide was started early, but due to a lack of improvement during two days, and in view of the bacteremia, a blood transfusion was given. A most remarkable improvement followed; the temperature became normal within a few days; ulcerations on the tongue began to heal; and the blood culture showed a sparse growth after the transfusion. The blood picture gradually returned to normal. The patient was well three weeks after onset of the illness and was discharged.

Case 2. S. K., a saleslady, age twenty-six years, was admitted to Mount Sinai Hospital (Service of Dr. B. S. Oppenheimer), July 5th, 1934, with a history of malaise, anorexia, fever and pain in the back for nine days before admission. Sore throat and gums were present for the past five days. There was no history of medication.

Physical examination: Examination revealed: swelling of the lower right eyelid; ulceration covered with black slough on the inner side left cheek; ulcerations about the teeth in upper and lower jaws; redness of the pharynx and tonsils.

On admission to the hospital, the blood picture was diagnostic. The hemoglobin, erythrocytes and platelets were normal. There was a marked leukopenia and neutropenia, with a relative lymphocytosis and monocytosis. A good prognosis was ventured in view of the marked monocytosis.

Course: The patient's temperature, which had been 102°F. on admission, gradually became normal. Intramuscular injections of liver extract (3 cc.) were given daily. The blood picture returned to normal after a week. Lesions in the mouth cleared up, and the patient was discharged after thirteen days. The results of blood examinations are shown in table 1.

One month later, in the Follow-Up Clinic, the patient was given 0.25 gram of pyramidon three times a day, for several days. During this period the blood picture remained unchanged.

Case 3. J. W., a male, fourteen years of age, was first seen by Drs. Clarence J. Cohen, of Flushing, New York, and Paul Chodack, of Brooklyn, New York

Symptoms began with an upper respiratory infection which apparently subsided in three days. The boy was able to get about when first seen, but chills, fever, sore throat, malaise, and loss of appetite set in two days later. He was treated with acetyl salicylic acid. He received no amidopyrine at any time. No ulcerations were present. The blood picture at this stage of the illness revealed 2,100 leukocytes, 1 per cent polymorphonuclears, 2 per cent eosinophils, 81 per cent lymphocytes, and 16 per cent monocytes.

TABLE 1

BLOOD EXAMINATIONS. CASE 2

DATE	LEUKOCYTES	NON-SEGMENTED NEUTROPHILS	SEGMENTED NEUTROPHILS	LYMPHOCYTES	MONOCYTES
1934		*per cent*	*per cent*	*per cent*	*per cent*
7–6	1500	4	1	80	15
7–7	2000	1	3	80	16
7–9	2800	8	17	64	10
7–10	3800	21	26	45	6
7–11	6600	25	33	35	6

Course: The condition became worse, and the temperature fluctuated reaching as high as 105°F. He became toxic and ulcerations appeared on the posterior part of the lip and on the gum margins. A blood examination made at that time revealed:

Hemoglobin	100 per cent
Erythrocytes	5,390,000
Leukocytes	3,700
Platelets	200,000
Neutrophils:	
Non-segmented	0.5 per cent
Eosinophils	6.0 per cent
Basophils	1.0 per cent
Lymphocytes	40.5 per cent
Monocytes	52.0 per cent

In view of the marked leukopenia and monocytosis, a diagnosis of leukopenic infectious monocytosis was made, and a good prognosis was given. About the twelfth day of illness, the ulcerations became more pronounced and extended from the gums to the inner side of the cheek; these necrotic areas were covered with a whitish slough. On the following day, patient began to show signs of improvement; the leukocytes increased to 4,500, and 20 per cent neutrophils appeared in the peripheral blood. The necrosis healed within the next few days and the patient was apparently well on the fifteenth day. Nevertheless, the blood picture did not completely return to normal until about three months after the onset.

Leucopenic infectious monocytosis with recovery

(*a*) *History of taking amidopyrine prior to onset*

Case 4. Mrs. M. D., a trained nurse, thirty-four years of age, was first seen by Dr. M. Dolganos, of Jersey City, New Jersey, November 5, 1932. For any ache or pain the patient had taken amidopyrine, during the past few years. She complained of sore throat followed by swelling of nodes in the neck four years ago. Had an attack of grippe one year ago, at which time leukopenia was noted.

The present attack was somewhat more severe, and followed a cold. The second day of illness, she developed pain in the back of her neck where a few tender glands were found; no sore throat was present. The following remarkable blood changes were noted:

Hemoglobin	95 per cent
Erythrocytes	4,400,000
Leukocytes	1,800
Platelets	250,000
Neutrophils:	
Non-segmented	1 per cent
Lymphocytes	64 per cent
Monocytes	32 per cent

This case represented a mild form of agranulocytosis of the monocytic type. Rapid recovery took place within a week. The leukocytes rose to 11,000; granulocytes increased to 62 per cent; lymphocytes numbered 31 per cent; and the monocytes, 7 per cent.

Patient was comparatively well for two months. Then she complained of malaise, sore gums, and sore tongue; temperature was 100.4°F. Within the next few days the condition became somewhat worse. Local measures were used for the mouth lesions which presented typical necrotic ulcerations. Rapid improvement occurred as in the previous attack. The blood picture showed a marked leukopenia (leukocytes, 1,600), and monocytosis. The non-segmented neutrophils were 11 per cent, segmented neutrophils, 13 per cent, lymphocytes 59 per cent, and monocytes 17 per cent. The blood picture became normal one week after the onset.

Following this attack (which occurred in latter part of February, 1933), the patient's attention was called to the deleterious effects of amidopyrine and its possible connection with symptoms in her case. She discontinued this form of medication and has since remained apparently well.

Case 5. F. P., a housewife, fifty-four years of age, was admitted to Mount Sinai Hospital (Service of Dr. B. S. Oppenheimer). November 1, 1934, with a history of chills and fever one and one-half years ago; phlebitis in both legs, ten months ago; swelling and redness of knees and ankles, five and one-half

weeks ago; pain in right shoulder, fever, followed by pain in chest, and chills, five weeks ago. The patient had used amidopyrine moderately throughout these periods. Five days before admission she complained of malaise, chills, fever, sore throat, and exacerbation of arthritic pains.

Physical examination: Pharynx, red; tonsils, enlarged and inflamed; elliptical tender node at angle of left jaw; heart and lungs, negative; right ankle, indurated and swollen; right and left knees, swollen and tender. Blood examination revealed on November 3, 1934:

Hemoglobin	65 per cent
Erythrocytes	3,720,000
Leukocytes	2,400
Platelets	370,000
Neutrophils:	
Non-segmented	2 per cent
Lymphocytes	70 per cent
Monocytes	28 per cent

Course: The patient was treated daily with 2 c.c. of liver extract intramuscularly. Her temperature, which on admission had reached 104°F., fell gradually to normal within eight days. Improvement became apparent; her joints resolved and she was discharged after twenty-four days.

(*b*) *After anti-luetic treatment with arsenobenzol*

Case 6. J. G., a female, thirty-two years of age, was admitted to Mount Sinai Hospital (Service of Dr. B. S. Oppenheimer), February 23, 1934, with a history of sore on her lip three months previously, in addition to a positive Wassermann reaction. Treatment with bichloride of mercury and potassium iodide was started, followed by injections of thibismol alternating with 0.5 grams sulpharsphenamine. In all, the patient received 1.5 grams sulpharsphenamine in three weeks. She complained of diffuse abdominal pains and increasing jaundice, dark urine and acholic stools for three weeks prior to admission. The last injection of sulpharsphenamine was given five days before entry, when severe pain developed in the right lower jaw with fever (102°F.), and swelling of nodes in the neck.

Physical examination: Sclerae icteric; necrotic ulceration of gums in right jaw; tender cervical nodes (right); and liver, enlarged to umbilicus.

The blood picture was typical of monocytic agranulocytosis. A secondary anemia was also present.

Course: The patient was given one blood transfusion, February 24, 1932. Liver extract was given intramuscularly, for two weeks. She improved steadily, and blood picture gradually returned to normal. An unusual eosinophilia (up to 20 per cent) was noted the first week. Patient was last seen in Follow-Up Clinic, January 21, 1935, and was found to be apparently well.

Results of blood examinations are given in table 2.

Fatal cases of leukopenic infectious monocytosis

(*a*) *History of taking amidopyrine*

Case 7. Sister M. A., forty-four years of age, was admitted to Mount Sinai Hospital (Service of Dr. A. A. Berg), October 6, 1932, with a history of appendectomy in 1911, cholecystectomy in 1912, gastro-enterostomy in 1914, and an operation for adhesions in 1920. For about one year there had been a history of furunculosis, weakness, abdominal pains with alternating diarrhea and constipation which gradually became more severe. Many kinds of sedatives, especially amidopyrine, were taken during the past year. Sore throat, ulcers in the mouth, and marked weakness were present two months prior to admission. She complained of sore throat and tongue two days before admission.

Physical examination: Necrotizing ulcerations on both tonsils and oropharynx; heart, soft systolic blow over precardium.

TABLE 2

BLOOD EXAMINATIONS. CASE 6

DATE	HEMOGLOBIN	ERYTHROCYTES	LEUKOCYTES	PLATELETS	GRANULOCYTES		EOSINOPHILS	LYMPHOCYTES	MONOCYTES
					Non-segmented	Segmented			
1932	*per cent*	*millions*			*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
2–24	57	3.45	2,500	320,000	3	7	7	45	38
2–25	76	4.89	2,400	320,000	12	6	8	34	39
2–26	78	4.50	1,900	450,000	8	8	20	40	24
3–3			3,900		3	34	6	50	6
3–14	74	4.09	6,050	330,000	5	43	4	35	11

Course: The patient became progressively worse. Ulcerations became widespread in the oral cavity; she ran a septic course with toxemia and fever. Two transfusions (400 c.c. each) and ten intramuscular injections of pentosenucleotide were given. Her temperature ranged from 100° to 105°F.; preagonally 107°. She died eighteen days after admission (October 24, 1932). No necropsy was performed.

Examinations of her blood are recorded in table 3.

(*b*) *History of taking atophan and amidopyrine*

Case 8. C. P., a male, accountant, forty-nine years of age. His past history was negative except for arthritis (ten years), for which he had taken a large amount of atophan (Service of Dr. Sara Welt).

Present illness began two weeks prior to admission, at which time the patient had a mild upper respiratory infection; eight days before entrance he experienced great fatigue. He subsequently developed a severe cough and expectorated a reddish sputum. His temperature range between 102° and 105°F.

and he took two "hexin" tablets (6 grains amidopyrine) one week after onset, but no other medication.

Physical examination: The man was acutely ill, cyanotic; no icterus; throat, negative. There were râles at the base of his right lung. Diminished breath sounds and dullness were present at the left base. There was a short systolic murmur which was heard at the left sternal border.

TABLE 3

BLOOD EXAMINATIONS. CASE 7

DATE	HEMOGLOBIN	ERYTHROCYTES	LEUKOCYTES	PLATELETS	GRANULOCYTES		LYMPHOCYTES	MONOCYTES
					Non-segmented	Segmented		
1935	*per cent*	*millions*			*per cent*	*per cent*	*per cent*	*per cent*
10–9	81	6.58	3,100	180,000	2	2	69	25
10–11	72	5.20	2,500	230,000			66	32
10–12	70	5.15	1,100	280,000	4	1	39	56
10–13	65	4.25	2,400	290,000			67	33
10–20	68	4.90	600	440,000			54	46

Laboratory data: Wassermann test, negative; sputum, pneumococcus, type VII; the blood picture on March 5, 1935 showed:

Hemoglobin	96 per cent
Erythrocytes	4,680,000
Leukocytes	600
Platelets	180,000
Neutrophils:	
Non-segmented	1 per cent
Myelocytes	1 per cent
Lymphocytes	52 per cent
Monocytes	44 per cent
Plasma cells	2 per cent

Course: The patient became progressively worse; he was toxic, irrational and stuporous. His temperature ranged between 102° and 105°F. He did not respond to treatment which consisted of four transfusions and intramuscular injections of liver extract, twice daily. Jaundice and necrosis of the gums were noted two days before death.

Necropsy: There was bronchopneumonia, fibrinous pleuritis, acute necrotizing esophagitis, multiple necrotic ulcerations of colon and rectum, splenomegaly, healed interstitial valvulitis of mitral and aortic valves. The vertebral bone-marrow was dark red. Among the essential microscopic findings were the following:

Lungs: The pleural and interstitial capillaries were intensely hyperemic. There was evidence of hemorrhage into the alveoli. Many of the latter con-

tained lymphocytes and large endothelial cells, some with ingested pigment. The bronchi were filled with amorphous, smudgy, basophilic material which enmeshed various cellular elements (erythrocytes, endothelial cells and lymphocytes).

Liver: Kupfer cells seemed to be more numerous than normally. Scattered throughout the sinusoidal spaces were occasional large cells with pale, slightly basophilic cytoplasm and round or oval shaped nuclei. Some of these cells

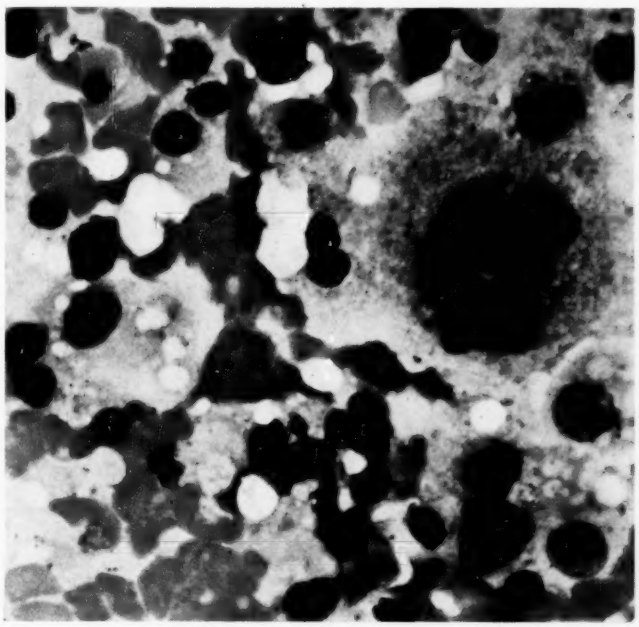

FIG. 1. SMEAR OF STERNAL BONE MARROW (CASE 8). MEGAKARYOCYTE

Note increase of large vacuolated cells, reticulo-endothelial in type, and moderate number of lymphocytes.

contained ingested erythrocytes. The portal areas were infiltrated with lymphocytes and large round cells.

Spleen: The trabeculae were prominent; the malphigian follicles were large. At the periphery of the latter, and in the cords, large cells with irregular nuclei were seen, as well as erythrocytes and lymphocytes. The large cells were identified as monocytes in smears made from the fresh spleen.

Bone-Marrow: There seemed to be a wide variation in the cellular structure between the sternal and vertebral and femoral bone-marrow. The latter was fatty and fairly well depleted of cells. The cells in sections of the vertebral

marrow were more numerous but a definite differential count could not be made. There was considerable difficulty in identifying various types of cells in sections which were fairly well stained with the Giemsa reagent. Megakaryocytes appeared in normal numbers. A few eosinophilic myelocytes with an occasional polymorphonuclear eosinophil and numerous plasma cells could be easily identified. Plasma cells and lymphocytes appeared to be numerous. There were large cells often vaguely outlined with large slightly irregular nuclei. These appeared to correspond with the large reticuloendothelial cells found in the impress preparations of bone-marrow (fig. 1). The normoblasts were present in moderate numbers. The bone-marrow appeared congested from the transfused erythrocytes. The following differential count on 500 cells was made from smears:

Neutrophils:	
Segmented	1 per cent
Eosinophils	1 per cent
Myeloblasts	2 per cent
Myelocytes:	
Neutrophilic	2 per cent
Eosinophilic	2 per cent
Macrophages (reticulo-endothelial)	26 per cent
Lymphocytes	47 per cent
Plasma cells	6 per cent
Normoblasts	13 per cent

No monocytes could be identified as such in the smears made directly from the bone-marrow, unless one considers the reticulo-endothelial or reticulum elements as the progenitors of the monocytic cells. In this connection, the study of the marrow with supra-vital methods proved of importance in identifying the monocytic cells. Such a differential count was made by Dr. L. A. Erf:

Neutrophils:	
Non-segmented	12 per cent
Myelocytes:	
Neutrophilic	8 per cent
Eosinophilic	8 per cent
Monocytes	58 per cent
Lymphocytes	14 per cent

With supravital stain the large vacuolated reticulo-endothelial cells (fig. 1) appeared to be typical monocytes.

(*c*) *Recurrent Agranulocytosis. History of taking amidopyrine*

First attack, monocytic; second attack, lymphocytic

Case 9.* Mr. L. G., thirty-seven years of age, restaurant owner, complained of swaying sensations for about one year. He took amidopyrine occasionally,

* The authors are indebted to Dr. M. Bruck for permission to publish this case.

without relief. During the last week in August, 1932, he complained of headache and dizziness, sore throat, slight soreness of the gums. His temperature was 103°F.

Physical examination: Negative, except for slight redness of the gums and pharynx and slight abdominal tenderness. The presence of a leukopenia and a marked monocytosis were revealed by the following blood examination, August 30, 1932:

Hemoglobin	102 per cent
Erythrocytes	5,460,000
Leukocytes	2,950
Platelets	300,000
Neutrophils:	
Non-segmented	6 per cent
Segmented	11 per cent
Lymphocytes	49 per cent
Monocytes	33 per cent
Myeloblasts	1 per cent
Degenerative Index	90 per cent

Applications to the gums with bismuth salicylate and glucose apparently relieved the local condition. He still complained of swaying sensations which, however, were less severe than previously. The patient's blood picture returned to normal with improvement in his general condition.

Approximately six months later, he developed a sore throat. At the advice of friends, a laryngologist was consulted and x-ray treatment was advised. His condition became considerably worse; extensive necrosis appeared on both tonsils, extending posteriorly to the pharynx. A blood picture typical of agranulocytosis was present at this time, (January 26, 1933):

Hemoglobin	80 per cent
Erythrocytes	4,240,000
Leukocytes	1,200
Platelets	160,000
Lymphocytes	96 per cent
Monocytes	4 per cent

Pentose-nucleotides were given daily, and a transfusion was given January 27, 1933. His condition, however, grew progressively worse. His temperature, which remained high throughout the course of the disease, was 106°F. on last day of illness. He became very apprehensive and dyspnoeic and died.

(*d*) *After treatment with bismuth: Hematological recovery but terminal pneumonia*

Case 10.* A. M. K., a female, twenty-three years of age, was admitted to the Willard Parker Hospital, June 27, 1934, with an onset of sudden chills,

* The authors are indebted to Dr. Lawrence W. Smith for permission to publish this case.

vomiting, fever, and difficulty in swallowing, two days before admission. These complaints followed a course of intramuscular bismuth injections instituted because of a positive Wassermann test.

Physical examination: Temperature 105°F. Membranous exudate involving tonsils, post-pharynx, and part of soft palate.

Course: Temperature: septic in type. Intramuscular liver extract and two transfusions were given. She developed pneumonia of the right lung, and died July 3, 1934, (seventh day of illness).

Comment: Agranulocytosis was suspected when the leukocytes numbered 1,550, with 13 per cent neutrophils. The following day (June 30, 1934), the blood picture showed some improvement.

Leukocytes	3,400
Neutrophils:	
Non-segmented	4 per cent
Segmented	1 per cent
Myelocytes	3 per cent
Lymphocytes	22 per cent
Monocytes	70 per cent
Degenerative Index	100

Although no clinical improvement was apparent, the blood count, July 2, 1934, presented an unusual increase in neutrophils:

Hemoglobin	92 per cent
Erythrocytes	5,080,000
Leukocytes	5,050
Platelets	180,000
Neutrophils:	
Non-segmented	65 per cent
Segmented	9 per cent
Lymphocytes	16 per cent
Monocytes	9 per cent
Plasma cells	1 per cent
Degenerative Index	100

The day of patient's death, the leukocytes reached 12,000, with 85 per cent neutrophils.

Pathological diagnosis: Agranulocytic angina (atypical); bilateral bronchopneumonia; petechiae of pleura, renal pelves and stomach; toxic hepatitis; erosion of cervix uteri.

Microscopic appearance of bone marrow: This was hyperplastic. Smears indicated a marked predominance of neutrophilic myelocytes, eosinophilic myelocytes, and myeloblasts; a few mature neutrophils and nucleated erythrocytes were also present.

(*e*) *After arsenobenzol: Hematological recovery but with terminal pneumonia*

Case 11. F. L., a female, thirty-four years of age, was admitted to Mount Sinai Hospital (Service of Dr. Leo Kessel), December 5, 1932, with a history of two operations for fistula in ano. On last admission, two months before, Wassermann reaction was positive. The patient was referred to the Out-Patient Department, and there received three intramuscular injections at weekly intervals, following which three intravenous injections of arsenobenzol were given. Malaise, body pains, chills and swelling of feet developed after the first intravenous injection; similar reaction occurred after the second. The third, five days before admission, resulted in a chill, with temperature at 104°, followed by a sore throat which continued up to the time of admission.

Physical examination: The patient was acutely ill, pharynx injected, pillars swollen, and with tonsils covered with a thick, greenish, gangrenous, sloughing

TABLE 4

BLOOD EXAMINATIONS. CASE 11

DATE	LEUKOCYTES	GRANULOCYTES		LYMPHOCYTES	MONOCYTES
		Non-segmented	Segmented		
1932		*per cent*	*per cent*	*per cent*	*per cent*
12–6	3,700	1		41	56
12–8	5,000	2		41	56
12–12	6,150	65	2	20	12
12–13	6,450	71	6	16	7
12–14	12,500	71	9	7	7
12–15	13,650	56	7	18	9
12–16	14,300	59	25	5	8

exudate. There were ulcerations on gums, also bilateral cervical adenopathy with a soft blowing systolic murmur at the apex.

Course: The patient was given four transfusions. Her temperature remained high, ranging between 101° and 105°F.: tonsils sloughed out and ulcerations spread to the posterior pharynx and epiglottis. Her blood condition improved, and on the twelfth day there were 67 per cent granulocytes when she developed pneumonic signs. The patient's condition became progressively worse. She died on the sixteenth day after admission, as a result of the overwhelming infection.

Blood cultures were negative. The results of blood examinations are shown in table 4.

Necropsy: Multiple lung abscesses; putrid empyema (right) following rupture of subcortical abscess of right upper lobe of lung; necrotizing tracheo-bronchitis; pulmonary congestion and edema; hemorrhagic proctitis; parenchymatous degeneration of liver, kidneys, and heart; subacute hemorrhagic cystitis; chronic salpingo-oöphoritis and fibrous pelvic adhesions.

Microscopic findings. Lungs: Section showed several small abscesses with complete destruction of parenchyma; many bronchi and bronchioles were severely damaged and ulcerated, and were filled with a polynuclear exudate. There were foci of suppurative bronchopneumonia about the abscesses. The suppurative process seemed to originate from the bronchi. Small bluish-staining foreign bodies were demonstrable in the center of some of the suppurative areas. The polynuclears were present not only in the abscess cavities

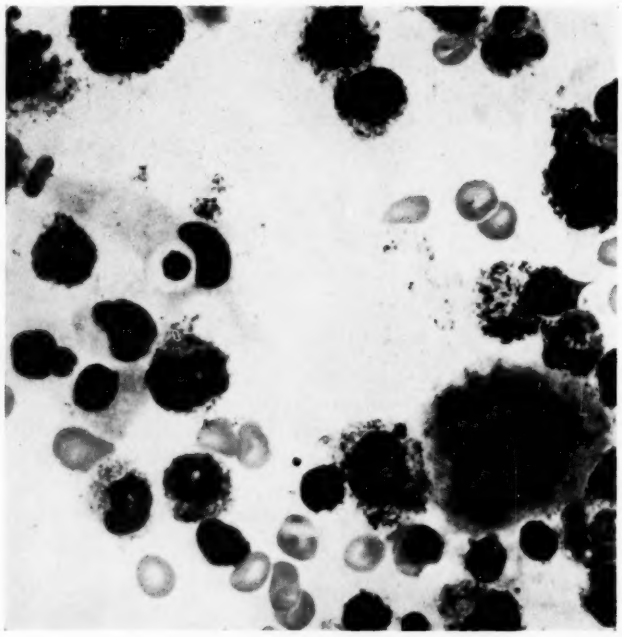

FIG. 2. SMEAR OF STERNAL BONE MARROW (CASE 11). MARKED INCREASE OF MYELOCYTES, FEW NEUTROPHILIC POLYNUCLEAR CELLS, MYELOBLASTS, MEGAKARYOCYTE AND MACROPHAGE WITH INGESTED LYMPHOCYTE

but also in the exudate in the bronchi and in the walls of severely damaged bronchi.

Liver: Section showed marked parenchymatous degeneration and fatty change. The latter was most marked at the periphery of the lobule. There were isolated polynuclear cells seen in the severely degenerated areas. The Kupfer cells were swollen and prominent, their number being perhaps slightly increased.

Spleen: There was marked congestion, edema, and many hyaline areas. The follicles were diminished in size and number. Small thrombi were present

in some of the smaller veins. Pulp was cellular and contained an occasional polynuclear cell. Smears made from the spleen showed numerous reticulo-endothelial and plasma cells.

Bone marrow (fig. 2): The predominating cells were neutrophilic myelocytes. A few polynuclear neutrophils, myeloblasts and megakaryocytes were also present.

Lymph nodes: Sections through several cervical lymph nodes showed marked sinus catarrh with foci of reticulo-endothelial cells in the sinuses amongst which were polynuclear cells.

Leucopenic infectious monocytosis followed by a persistent leukopenia and monocytosis

(*a*) *No history of using drugs*

Case 12. J. F., 45 years of age, had been perfectly well except for an infection of the tendons of the hand, for which he was treated surgically in 1910.

Present history: In May, 1925, he was admitted to Beth David Hospital, where Dr. Leo Buerger performed a nephrectomy for calculous pyonephrosis. Ten days after the operation there was a large hemorrhage in the wound which had shown no signs of healing. Patient developed marked weakness and pallor and at times lapsed into a stupor.

A blood examination made on May 21, 1925, resulted as follows:

Hemoglobin	52 per cent
Erythrocytes	3,120,000
Leukocytes	1,400
Platelets	210,000
Neutrophils:	
Non-segmented	11 per cent
Eosinophils	5 per cent
Basophils	1 per cent
Myelocytes:	
Neutrophilic	10 per cent
Myeloblasts	7 per cent
Lymphocytes	50 per cent
Monocytes	16 per cent

The secondary anemia was not unexpected, but the presence of an unusual percentage of myelocytes and myeloblasts and monocytes, was suggestive of a leukemia.

Physical examination: Nothing abnormal was noted, except for a slight ulceration on the tongue which developed after the hemorrhage.

After a transfusion of 500 cc. of unmodified blood, the patient improved remarkably. The wound began to heal quite rapidly, and the patient was able to leave the hospital two weeks later.

Periodic blood examinations were made for a period of ten years after he

left the hospital, all indicating a persistent leukopenia, lymphocytosis, and monocytosis (table 5).

(*b*) *Recurrent, with history of taking pyramidon*

Case 13.* Mrs. S. B. S., forty-eight years of age, had been in good health except for frequent colds until the first attack, which started about January 1, 1932. Symptoms began with sore throat, followed by laryngitis. A few days later this became very severe with dyspnea and dysphagia. Her temperature rose daily to 102°F., and occasionally higher.

TABLE 5

BLOOD EXAMINATIONS. CASE 12

DATE	LEUKOCYTES	GRANULOCYTES			MYELOBLASTS	LYMPHOCYTES	MONOCYTES
		Non-segmented	Eosinophils	Myelocytic neutrophils			
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
5–21–25	1,400	12	5	10	7	50	16
6– 1–25	3,400	4	9	3	2	67	15
6–15–25	3,000	8	2	2		63	25
9–18–25	3,600	9	4			61	26
10–31–25	2,700	16				62	21
3–15–27	3,000	11	8			68	9
4–19–29	3,800	1	5			65	15

Physical examination: Negative, except for ulcerations on one of the vocal chords, and edema of the glottis.

Agranulocytosis was suspected by her attending physician, Dr. H. R. Miller. This was confirmed by the blood count:

Hemoglobin	82 per cent
Erythrocytes	5,010,000
Leukocytes	1,500
Platelets	520,000
Neutrophils:	
Non-segmented	2 per cent
Eosinophils	1 per cent
Basophils	1 per cent
Lymphocytes	77 per cent
Monocytes	8 per cent
Histiocytes	10 per cent

Course: Annoying symptoms from the laryngitis lasting months and did not clear up until patient coughed up a small piece of cartilage. The temperature

* The authors are indebted to Dr. H. R. Miller for permission to publish this case.

then became normal and the patient was apparently well after three and one-half months. The blood picture remained unchanged; the leukopenia and monocytosis persisted until the second attack which began on December 21, 1934. The initial symptom was a slight upper respiratory infection, and again laryngitis supervened. The temperature ranged from 101° to 102°F. The symptoms subsided at the end of four weeks.

The blood picture found at the onset of this attack, was as follows:

Hemoglobin	80 per cent
Erythrocytes	4,460,000
Leukocytes	4,700
Platelets	240,000
Neutrophils:	
Non-segmented	1 per cent
Eosinophils	3 per cent
Lymphocytes	54 per cent
Monocytes	42 per cent

The blood examination made May 11, 1935, about four months after the patient recovered still showed a tendency to leokopenia and neutropenia:

Leukocytes	5,800
Neutrophils:	
Non-segmented	8 per cent
Segmented	15 per cent
Eosinophils	4 per cent
Basophils	2 per cent
Lymphocytes	58 per cent
Monocytes	13 per cent

History of drugs: At the onset of the second attack the history of taking drugs was investigated. For several years the patient had been in the habit of taking such drugs as amidopyrine, midol, aspirin, and luminal for headache or insomnia, and continued to take them throughout the first attack of laryngitis. These drugs were taken at intervals until the second attack on December 21, 1934, after which they were discontinued. The blood picture, however, has not presented any tendency to return to normal, which possibly indicates some unusual type of bone-marrow disturbance. At present, the patient is apparently well, except for occasional headaches.

(c) *Recurrent case without the history of drugs*

Case 14.* Mother L., fifty years of age had her first attack in September, 1934, when she noticed painful ulcerations on the mucous membrane of her right cheek, lower lip and left margin of the tongue. There was also general malaise, loss of weight, and slight fever.

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* The authors are indebted to Dr. E. Secondari for permission to publish this case.

The blood examination revealed a slight secondary anemia (hemoglobin, 75 per cent and erythrocytes, 3,460,000), with no leukopenia (leukocytes, 6,400), but there was a marked neutropenia:

Neutrophils:	
Segmented	12 per cent
Lymphocytes	86 per cent
Monocytes	2 per cent

The ulcerations responded to local treatment and patient recovered.

In February, 1935, an attack of acute pharyngitis was followed by otitis media and then mastoiditis. Mastoidectomy was performed immediately. The blood examination was essentially the same as before. A count on February 25, 1935, presented a marked leukopenia of 3,750 leukocytes with neutrophils being depressed to 2 per cent and lymphocytes 90 per cent and monocytes 6 per cent. Again, the patient made an uneventful recovery.

TABLE 6

BLOOD EXAMINATIONS. CASE 14

DATE	HEMOGLOBIN	ERYTHROCYTES	LEUKOCYTES	PLATELETS	GRANULOCYTES		LYMPHOCYTES	MONOCYTES
					Non-segmented	Segmented		
1935	*per cent*	*millions*			*per cent*	*per cent*	*per cent*	*per cent*
5–4	60	3.00	4,000	250,000	1	1	86	11
5–6*	82	4.15	4,100		4	4	62	29
5–9			3,800		4	3	86	7
5–21			5,100		1	5	81	12
6–1					1	2	87	9

* After transfusion.

The third attack began April 16, 1935, with fever and marked dyspnea and sore tongue, where an ulceration was present. The laryngeal examination revealed severe edema of the larynx; liver and spleen were palpable.

The blood picture again was similar to that found in previous attacks, except for the increase in monocytes.

Two transfusions and intramuscular injections of liver extract, twice daily, were given. The patient improved rapidly after a small piece of cartilage was coughed up. Since recovery, the blood picture has remained unchanged (table 6).

DISCUSSION

The present report of fourteen cases of leukopenic infectious monocytosis may serve to indicate the extent of variation which

may occur in connection with the onset, course, and blood picture in this form of agranulocytosis.

Symptoms and course

The majority of the cases started like an infectious process, with malaise, chillness, weakness, and anorexia. In some instances the symptoms were mild, simulating an upper respiratory infection or a slight sore throat. Soreness of the tongue and gums were not infrequent after the onset. Symptoms of a very severe nature were also encountered, such as stupor, marked toxemia, and pyrexia, associated with extensive ulcerations of the pharynx, tongue, or gums. The presence of necrotizing ulcerations on the tongue and gums exclusively, was a characteristic feature of the monocytic form of agranulocytosis. Ulcerations were localized to the larynx alone in cases 13 and 14. No definite necrotic manifestations were found in case 5, although the patient had some congestion of the pharynx. Cervical lymphadenopathy was present in three instances; these were small non-tender glands and seemed to be associated with the oro-pharyngeal infection (cases 3, 4, and 5).

The greater number of instances recorded in the literature in addition to our own, were of an acute nature, the duration of the attack varying from one week to two months. Recurrent cases of the monocytic variety have been reported by Goldenberg,[16] Blumer,[2] Fogh and Lorenzon[13] and de Vries.[33] Cases 4, 9, 13, and 14, in the present series, are examples of this type. In case 9, the first attack was distinctly monocytic, whereas the second, which proved fatal, was lymphocytic agranulocytosis.

An unusual persistence of leukopenia, neutropenia, and monocytosis, was revealed in cases 12, 13, and 14, after the acute attack had subsided. In fact, the blood picture did not return to normal after apparent clinical recovery. More or less, similar cases of chronic or cyclic agranulocytosis have been reported by Rutledge, Hansen-Prüss and Thayer,[29] Doan,[8] Conner,[5] and Staley.[31] In these cases, as well as our own, recurrent attacks of agranulocytosis were not infrequent.

Etiology

The cause of agranulocytosis has not been satisfactorily explained up to the present time. Three main factors have been suggested, these being: (1) abnormal disturbance of the bone-marrow; (2) unusual septic processes; and (3) the influence of drugs.

(*1*) *Abnormal disturbance of the bone marrow:* In many of the reviews as well as in the numerous reports of individual cases of agranulocytosis much stress has been laid on the underlying constitutional defect of the bone marrow, either of a transitory or persistent nature. Fitz-Hugh and Krumbhaar,[12] have compared agranulocytosis with pernicious anemia, and consider a lack of the maturation principle for leukocytes an important etiological factor. Still others have recently pointed to the allergic nature of this disease; this may apply to certain cases which are sensitive to drugs or possibly bacterial toxins. In general, these various theories can be applied only to certain selected cases. The constitutional defect may be revealed by the presence of a chronic leukopenic state. In others, the defect becomes manifest during the course of slight or severe intercurrent infection.

(*2*) *Unusual septic processes:* Relation of infection to agranulocytosis has been a disputed point. The question resolves itself into whether the infection or changes in the bone marrow appear first. Considerable proof has been presented in a large number of cases in which the bone-marrow is first affected, resulting in leukopenia and neutropenia, with infection ensuing. In cases 12, 13, and 14, as well as others reported in the literature, leukopenia and neutropenia preceded and followed the attacks of agranulocytosis. In many instances, the reverse has been found, namely, sepsis first, and rapid deterioration of the bone-marrow later.

No definite causation except that of infection is apparent in some. Here one must assume that some specific bacterial invader or an unknown virus with a selective affinity for the bone marrow is present. This may also manifest itself by a focal infection in the oro-pharynx or elsewhere, with the elaboration of toxins which depress the bone marrow. In this connection, the experiments of Dennis[7] may be cited. He has succeeded in

producing leukopenia and granulocytopenia in rabbits, by placing capsules containing cultures of streptococci and staphylococci in the peritoneal cavities.

In some cases of monocytic agranulocytosis the invading organism may possibly be a specific one and may be related to the unknown causative agent of infectious mononucleosis, which is commonly of the lymphoid type and rarely monocytic. In infectious mononucleosis, leukopenia or leukocytosis may be present at the onset. Case 3 resembles the rare monocytic type except for the persistent leukopenia. However, the relation of this disease to infectious mononucleosis or monocytic angina will remain in doubt until the actual underlying cause of these conditions is ascertained.

(*3*) *Relation to drugs:* History of taking drugs prior to the onset of symptoms was elicited in nine of the fourteen cases of monocytic agranulocytosis reported in this paper and in seven cases previously published.[27] Pyramidon was used in small or large dosage by seven of the patients. In one case it was taken as a headache tablet one week after the onset. Arsenobenzol played an important rôle in the development of the condition in two cases reported here and in one case in a previous report. Bismuth played a part in one case. Atophan was taken in large quantities for arthritis in case 8. It is interesting to note that one of our patients (case 1) was given allonal on six different occasions, while the condition was in the active state, but it seemed to have no influence on the favorable progress of the disease.

The actual radical responsible for the myelotoxic action has not been definitely established. Kracke[22] first considered the benzene ring or benzamine radical, as responsible; but in a more recent communication, he emphasized the easily oxidizable benzene ring into the quinone derivative, as the etiological agent. Herz[17] attributed the action not to the benzene, but to the pyrazalon ring.

Blood picture

It is noteworthy that marked monocytosis in certain cases of agranulocytosis has received slight attention. Dudel[9] reported

a case of agranulocytosis with marked monocytosis and directed attention to the fact that, possibly, it represented another form of the disease and that it had an unusual good prognostic import. Lichtenstein[24] pointed to the importance of two main features of the blood picture with reference to prognosis, namely: the presence of neutrophils and monocytes. In his fourteen cases of agranulocytosis with absence of monocytes, recovery occurred in only one. Of thirteen other instances of agranulocytosis, in which monocytes were present, five recovered. Lichtenstein[24] believed an absolute count of over 100 monocytes to be prognostic of recovery in agranulocytosis. Boch and Wiede[3] likewise consider a marked monocytic reaction to be of good import.

The most striking hematological feature of these cases of agranulocytosis is not only the relative increase in the percentage of monocytes, but in some cases, an actual absolute increase. Dameshek[6] has suggested a reticulo-endothelial hyperplasia as the underlying cause of this phenomenon. This has, in a sense, been confirmed by the microscopic study of sections of the liver, spleen, and bone marrow in the post mortem material from two of our cases.

The monocytes correspond to the large mononuclear and transitional cells first described by Ehrlich.[10] They are larger than the neutrophils, and present the characteristic irregular, rounded "u" or "s" shaped, finely reticulated nucleus. The cytoplasm usually stains evenly, and contains a few discrete azure granules. Oxidase positive granules can be demonstrated in the cytoplasm with the proper stains. Supravital staining by means of neutral red and Janus green B, brings out the characteristic features of this type of cell, namely: the evenly distributed and equal-sized vacuoles (so called "rosette"), and a few evenly distributed small mitochondria. They move very slowly when observed in the warm box. In addition to these cells, large clasmatocytes or macrophages may be found in the blood, obtained from the lobe of the ear, at times up to 10 per cent or more. Apparently, these are more numerous in the tissues; as many were present in large numbers in pneumonic areas in case 8.

The monocytosis appears early in this form of agranulocytosis.

It may increase slightly at first. During the stage of recovery they diminish in percentage as the neutrophils begin to appear in the blood stream. In the two fatal cases (cases 7 and 8) the percentage of monocytes remained constantly high (up to 50 per cent). Other constituents of the blood showed marked variations at the onset. The hemoglobin and erythrocytes were usually normal. They were somewhat reduced in cases 6 and 11, as a result of arsenobenzol treatment for lues. The platelets were normal in number. The leukocytes varied from 600 to 4,000. The granulocytes were entirely absent in the second attack of case 9, and varied from 1 to 17 per cent in other cases.

Treatment

No special therapy is indicated in the mild cases; they seem to clear up spontaneously. Local treatment to the lesions, such as Lugol's solution, 10 per cent salvarsan in glycerine, or a bland mouth wash, may be used.

In the more severe cases pentose-nucleotide (Jackson[21]), 10 cc. twice a day, intramuscularly; or liver extract (Foran, Sheaff and Trimmer[14]), twice daily similarly or intravenously injected, represent two main methods in vogue for stimulating the bone-marrow. Adenine or guanadine sulphate have been suggested by Reznikoff.[26] Transfusions should be given in addition to this medication, or it may be given alone as a supportive measure. Radiation to the long bones, according to Friedmann's[15] method has also been employed. Nevertheless, transfusion seems to be the most desirable of all the methods so far known. Liver extract therapy appears to be followed by better results and less reaction than pentose-nucleotide or adenine sulphate. These forms of medication are all nonspecific, and seem to be of no value in the more malignant types of the disease. Pentose-nucleotide and transfusions were administered without effect in case 7; and liver extract and transfusions were unsuccessful in case 8. Both of these patients remained leucopenic and monocytotic throughout the course of the disease. In the other cases a spontaneous tendency toward improvement was observed; convalescence was hastened by blood transfusions.

Prognosis

In our previous report of eight cases, we expressed the opinion that recovery occurred in the monocytic type of agranulocytosis in spite of the fulminating and severe appearance of the disease. Following the observation of additional cases, however, it has been apparent that some patients may die.

A survey of the literature to the end of 1934 indicates recovery in sixty-five per cent of cases with marked monocytosis. In our own fourteen cases in the present series and the eight previously reported, five have died and seventeen have recovered (seventy-seven per cent). Two of the five made an hematological recovery, but succumbed to pneumonia, probably contracted during the leukopenic phase.

SUMMARY

(1) A classification of agranulocytosis, based on hematological findings, is suggested.

(2) We have presented and discussed fourteen additional cases of leukopenic infectious monocytosis (agranulocytosis with marked monocytosis).

(3) Leukopenic infectious monocytosis is apparently an infectious condition. A history of taking amidopyrine may be regarded as a predisposing factor in some of our cases; in others, atophan, bismuth, or arsenobenzol may have been responsible.

(4) The principal symptoms are mild or severe infection with necrotic ulcerations on mucous membranes. The tongue and gums are usually involved. The course may be acute, recurrent, or chronic.

(5) There is a leukopenia varying from 900 to 4,000 cells associated with a monocytosis at the onset of the disease. In three cases in our group a leukopenia, neutropenia and monocytosis persisted after the initial attack.

(6) The prognosis is more favorable in agranulocytosis accompanied by monocytosis. In a series of twenty-two cases so far observed, seventeen patients have recovered (seventy-seven per cent).

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THE CUTANEOUS REACTION TO AVIRULENT TUBERCLE BACILLI

(1) Reaction to Fine and Coarse Suspensions

(2) Reaction to Suspensions Prepared from Cultures on Mediums Containing Crystal (or Gentian) Violet

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Studies with tubercle bacilli present many complicated phases[4] covering a wide latitude of possibilities, which are almost individual for these organisms and which at times make deductions difficult unless exacting quantitative studies are pursued[14] and limitations are defined before drawing practical conclusions.[8] Thus, the bacilli will grow on a wide range of mediums, though only complete nutrients such as potato, egg, blood, or certain tissues, will support the growth of small numbers of bacilli;[3,12] likewise, heavy plantings on good nutrients may grow apparently less profusely than smaller plantings;[4] and, massive infections in experimental animals will lead to the deduction that the spontaneous disease is usually a bacteraemia, which is contrary to fact.[8]

With the increased interest in avirulent strains of tubercle bacilli aroused by the extensive practical use of an avirulent bovine tubercle bacillus (Bacillus Calmette-Guerin) for vaccination, it became even more pertinent that one define avirulence and that one note further the reaction of these avirulent tubercle bacilli as contrasted with virulent tubercle bacilli and with saprophytic acid fast bacilli (morphologically similar to tubercle bacilli but biologically readily differentiated). In a study just completed,[7,9] avirulent human or bovine tubercle bacilli were defined as tubercle bacilli capable of developing on a good nutrient medium suited for the growth of this group of bacilli, at an

optimum temperature of about 37°C.; when injected intravenously in fine suspension into a species of animal (guinea pig) susceptible to the type of tuberculosis in question in relatively large amounts (1 mgm.), these avirulent bacilli are incapable of producing gross organic tuberculous changes; and, when injected intracutaneously, produce no more lesion than an equivalent amount of dead bacilli (about 0.01 mgm.) and show no evidences of multiplication, but ultimately become nonviable in the animal economy.

Much confusion may arise from variations in experiments, and it was for the purpose of standardizing this that the observations to be recorded in this paper were made. It is appropriate to call attention to the changes noted in using different animals for comparisons without realizing that certain variations in findings may occur on the basis of the individual reactions of the animals, particularly as concerns tubercle bacilli. A previous contact with the bacilli may result in the establishing of a decided relative immunity with coincident marked change in reaction.[7] It was for this reasion that carefully bred young male guinea pigs were used in these tests and wherever possible and consistent with technical details comparisons were made in the same animal, for which purpose intracutaneous testing was particularly adapted.

COMPARISON OF THE INTRACUTANEOUS REACTION TO COARSE AND FINE SUSPENSIONS OF AVIRULENT TUBERCLE BACILLI

In earlier reports, the method of preparing fine suspensions of tubercle bacilli were described[5] and in certain cases the advantages of coarse suspensions detailed.[15] In most cases, however, fine uniform suspensions possess decided advantages for quantitative study and uniform organic distribution following intravenous injection.[10] A study of the distribution of fine suspensions of particulate material injected intracutaneously (in small amounts) also showed a tendency for filtration concentration at the site of the needle outlet.[6] In the belief that coarse and fine suspensions of avirulent human or bovine tubercle bacilli might differ decidedly both as to the size and time of development of the local reaction produced by them when injected intracutaneously, a series of guinea pigs were given injections of graded

amounts of coarse and fine suspensions of these bacilli, using the same animal for test and recording the findings by photographic record at the same magnification for comparative purposes. In addition, the usual record measurements were made at regular intervals. In preparing the suspensions every effort was made to obtain a coarse suspension which would not be too coarse to pass through the needle (24 gauge) used for injection and which would show quite striking physical differences from the fine suspension. The coarse suspension was prepared in 0.9 per cent sodium chloride solution, added in small amount before grinding was begun to insure a lumpy final division of the bacillary masses, while the fine suspension was made by grinding first without the addition of any liquid, then with a few drops of 0.5 per cent sodium taurocholate and finally diluting with 0.9 per cent sodium chloride solution. The fine suspension was opalescent milky in appearance, showed no evidences of settling on standing for hours and even when centrifugated at low speed showed a marked tendency to remain in suspension, while the coarse suspension settled quite rapidly on standing and was visibly coarse when examined by transmitted light. The animals were injected intracutaneously (usually in triplicate in the same animal) with 0.2 cubic centimeter of a one milligram per cubic centimeter suspension. This suspension is best suited for the test since it produced a definite lesion upon injection, it could be prepared fairly accurately and could be used without introducing dilution factors which might present errors in interpretation. Guinea pigs were chosen for test since the avirulent human and bovine bacilli selected for these experiments had been proved avirulent for this animal. In spite of the fact that the suspensions were prepared in widely different states of physical division, only slight yet definite differences in reaction to these suspensions were noted. This difference is to be attributed to the physical state of division of the bacilli before injection because of the avirulence of the tubercle bacilli used and since in each case they were obtained from the same part of the same rapidly growing young culture (about three to four weeks old). In all cases, the reaction to the fine suspension of avirulent tubercle bacilli de-

veloped more rapidly with the earlier appearance of a larger nodule and a greater tendency to ulceration. The result is best noted in the accompanying illustration showing the results with an avirulent bovine strain (BCG) of tubercle bacilli. (See plate 1.) Identical results were noted for an avirulent human strain of tubercle bacilli injected intracutaneously into the guinea pig.

COMPARISON OF THE INTRACUTANEOUS REACTION TO AVIRULENT HUMAN OR BOVINE TUBERCLE BACILLI GROWN ON A GOOD NUTRIENT MEDIUM WITH AND WITHOUT THE ADDITION OF CRYSTAL (OR GENTIAN) VIOLET

Gentian violet and crystal violet have been variously used in mediums for growing tubercle bacilli. Although this dye, as originally recommended, [11, 13, 16] exerts a definite static effect, we have used Petroff's egg medium containing 1:10,000 gentian or crystal violet for perpetuating numerous laboratory strains by relatively heavy transplanting without noting any apparent striking effect on the cultures even over long periods of time of continual use. However, it has been suggested that such cultures may show variations in virulence,[2] especially when dealing with avirulent strains of tubercle bacilli. The reaction to the intracutaneous injection of graded amounts of avirulent human and bovine tubercle bacilli grown on egg mediums, with and without the addition of gentian or crystal violet, were studied.

Usually in making comparative intracutaneous tests, fine suspensions were prepared from a three to four weeks old young culture on inspissated egg yolk medium[5] and on Petroff's gentian violet[16] or crystal violet egg medium, and graded amounts (0.2 cc. of 10.0, 1.0, 0.1, 0.01, 0.001, or 0.0001 mgm. per cubic centimeter) were injected side by side in the skin of the guinea pig. Comparisons included avirulent strains of human and bovine tubercle bacilli grown on the two different mediums. Young male guinea pigs were used for test and all experiments were carried out in duplicate. It was found that there was no consistent gross difference in the amount or type of skin lesion produced by intracutaneous injection of the fine suspensions of bacilli whether grown on the inspissated egg yolk medium or on the inspissated

egg mediums containing 1:10,000 dilution of gentian or crystal violet. This amount of dye is capable of exerting a definite static influence on the growth of small plantings of these tubercle bacilli. The results are graphically illustrated in the appended pictures (plate 2). The skin reactions with avirulent human tubercle bacilli conformed as a whole to those occurring with the avirulent bovine tubercle bacilli in normal guinea pigs. In these animals definite palpable tubercle formation occurs with dilutions of fine suspensions of these avirulent human and bovine tubercle bacilli as low as 0.01 mgm. of bacilli (as obtained directly from cultures) per cubic centimeter.

The intravenous injection of fine suspensions of avirulent human and bovine tubercle bacilli in 1 or 5 mgm. amounts obtained from cultures on egg mediums grown with or without gentian or crystal violet also revealed no differences either in the organic reaction to these suspensions or to a change in pathogenicity of the tubercle bacilli.

Although there are admittedly fine quantitative chemical differences between human and bovine tubercle bacilli[1] when grown artificially for chemical analysis, the differences in the lesions produced by the intracutaneous injection of the dead human or bovine tubercle bacilli are not sufficiently evident to be grossly perceptible. (See plate 3, figs. 3 and 4) to permit their differentiation by such tissue reactions. Likewise, viable avirulent human or bovine tubercle bacilli cannot be differentiated from non-viable human or bovine tubercle bacilli by their gross tissue reactions in animals (guinea pigs). (See plate 3, figs. 1 and 2.)*

SUMMARY AND CONCLUSIONS

Fine suspensions of avirulent human or bovine (BCG) tubercle bacilli when injected intracutaneously in normal guinea pigs produce a more marked and slightly greater tissue reaction than coarse suspensions in equal amount of the same bacilli taken from the same culture.

* We are grateful to L. D. Miller for assisting with the technical phases of this study.

The reactions to the intracutaneous or intravenous injection of fine suspensions of avirulent human or bovine tubercle bacilli grown on gentian violet or crystal violet (1:10,000) egg mediums do not differ appreciably from the reactions to those grown on inspissated egg yolk or other egg mediums not containing these dyes, which are static for small plantings of tubercle bacilli.

The skin reactions to the intracutaneous injection of avirulent human tubercle bacilli were similar to those occurring to avirulent bovine tubercle bacilli (BCG) in normal guinea pigs. Definite palpable tubercle formation occurs with dilutions of fine suspensions as low as 0.01 mgm. per cubic centimeter.

Although there exist quantitative chemical differences between human and bovine tubercle bacilli, no noteworthy differences in the lesions produced by the intracutaneous injection of fine suspensions of chemically or heat killed virulent or avirulent human or bovine tubercle bacilli in normal guinea pigs was noted, so that differentiation by this means is not possible.

Likewise, viable avirulent human or bovine tubercle bacilli could not be differentiated from non-viable human or bovine tubercle bacilli by the gross tissue reactions produced in animals.

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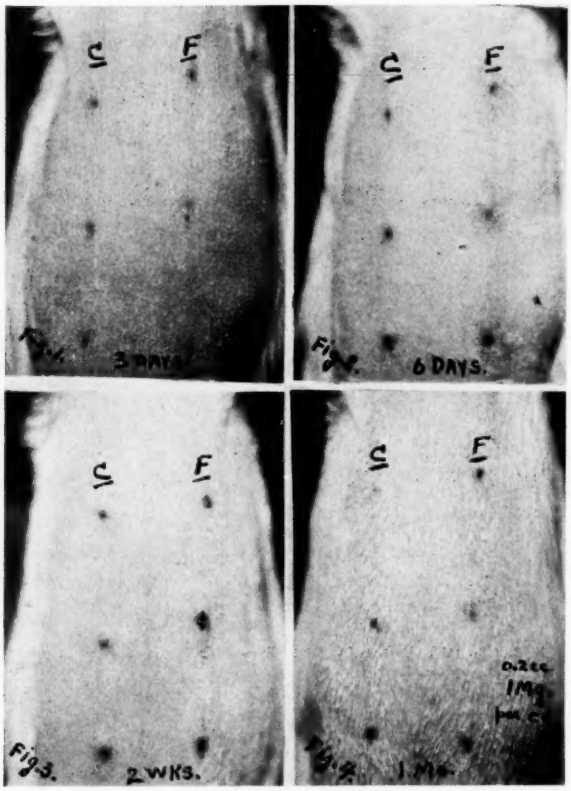

PLATE 1. COMPARISON OF THE INTRACUTANEOUS REACTION TO COARSE ("C") AND FINE ("F") SUSPENSIONS OF VIABLE AVIRULENT BOVINE TUBERCLE BACILLI (BCG)

Three injections of 0.2 cc. of the coarse ("C") suspension containing 1 mgm. per cubic centimeter of bacilli were given on one side and three of the fine ("F") suspension on the other. Figure 1 shows the results after 3 days; figure 2, after 6 days; figure 3, after 2 weeks; and figure 4, after 1 month. Note the more rapid and greater evolution of the lesions following the injection of the fine suspension.

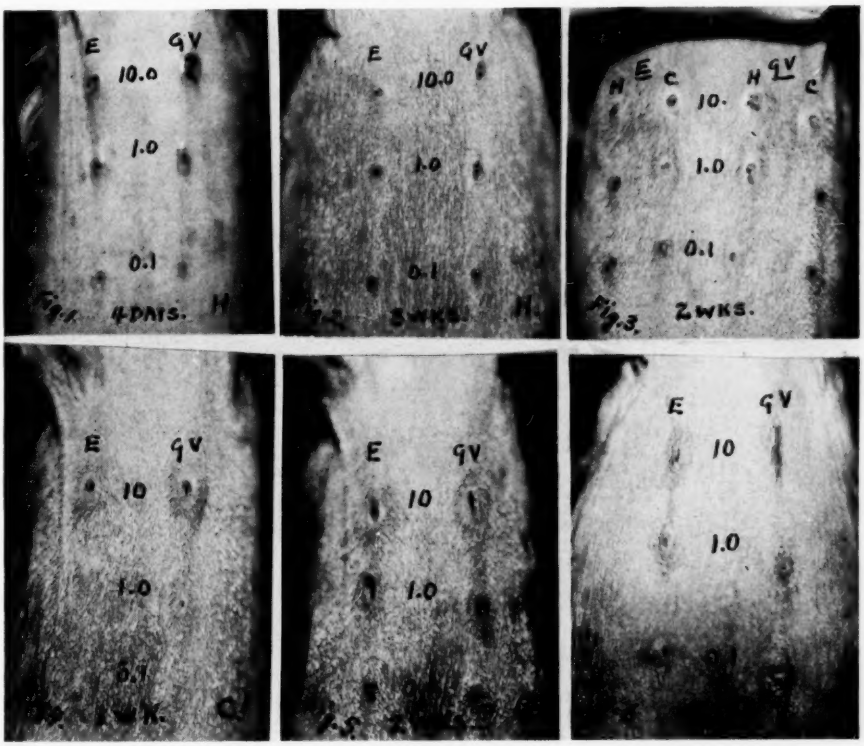

PLATE 2. COMPARISON OF THE INTRACUTANEOUS REACTION TO A FINE SUSPENSION OF AVIRULENT HUMAN AND BOVINE TUBERCLE BACILLI GROWN IN THE PRESENCE OF CRYSTAL (OR GENTIAN VIOLET)

Figures 1 (taken after 4 days) and 2 (taken after 3 weeks) show the results of graded (0.2 cc. of 10.0, 1.0, and 0.1 mgm. per cubic centimeters) injections of cultures of avirulent human tubecle bacilli grown on an inspissated egg yolk medium "E" and on an egg medium containing 1:10,000 gentian violet "G. V." Figure 3 (taken after 2 weeks) shows the reaction to graded injections (10.0, 1.0, and 0.1 mgm. per cubic centimeters) of avirulent human "H" and avirulent bovine "C" tubercle bacilli grown on egg medium "E" without and with gentian violet "G. V." Note the striking similarity of the lesions with the same amount of bacilli. Figures 4 (taken after 1 week), 5 (taken after 2 weeks), and 6 (taken after 5 weeks) show the reactions to graded intracutaneous injections of avirulent bovine tubercle bacilli (BCG) grown on egg medium, without gentian violet "E" and with gentian violet "G. V."

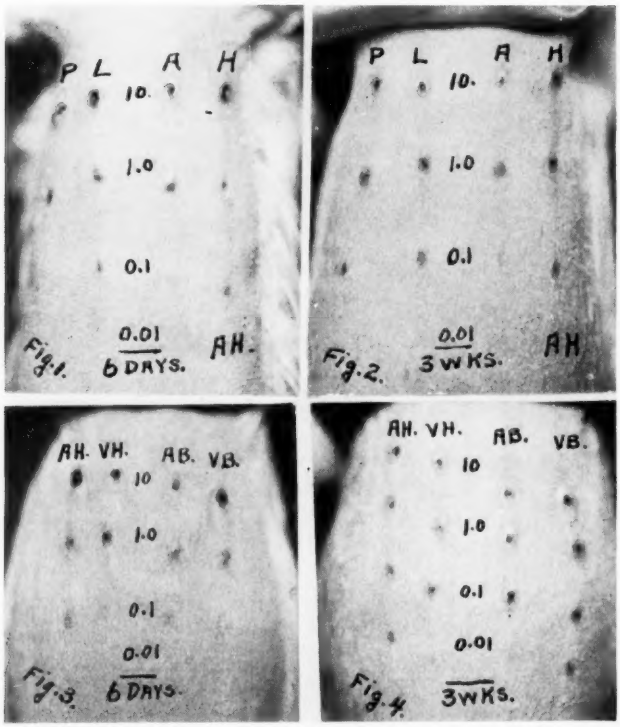

PLATE 3. FIGS. 1 AND 2. A COMPARISON OF THE INTRACUTANEOUS REACTION (FIGURE 1, TAKEN AFTER 6 DAYS, AND FIGURE 2, AFTER 3 WEEKS) TO LIVE "L" AND DEAD AVIRULENT HUMAN TUBERCLE BACILLI INJECTED IN GRADED AMOUNTS

The dead bacilli were killed with phenol "P", hexylresorcinol "H", or acetic acid "A". Note the similarity in the lesions regardless whether the bacilli were viable "L" or non-viable (P, A, H).

FIGS. 3 AND 4. A COMPARISON OF THE INTRACUTANEOUS REACTION TO NON-VIABLE AVIRULENT AND VIRULENT HUMAN AND BOVINE TUBERCLE BACILLI INJECTED IN GRADED AMOUNTS

In figure 3, hexylresorcinol was used to destroy the viability of the bacilli and the interval after injection was 6 days while in figure 4, acetic acid was used to destroy viability and the interval was 3 weeks after injection. The injections from left to right were an avirulent strain of human tubercle bacilli (A H), a virulent strain of human tubercle bacilli (V H), an avirulent strain of bovine tubercle bacilli (A B), and a virulent strain of bovine tubercle bacilli (V B).

HODGKIN'S DISEASE IN THE AGED*

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A case of Hodgkin's disease in a white male of seventy-seven years of age diagnosed at necropsy prompted me to review the literature and the records of St. Luke's Hospital during the past twenty years in an attempt to evaluate the incidence, clinical, and pathological manifestations of this disease occurring in the latter decades of life.†

This report comprises seventy-four cases which have been proved either by biopsy or necropsy, and whose clinical histories have been available, one of which is reported in detail because of his advanced age, being seventy-seven when first seen. As the criteria for a diagnosis of Hodgkin's disease are not standardized and marked morphological variations may occur in different cases and in different stages of the same case, we have rejected those cases lacking a polymorphocellular structure. These cases include those having histologically a granulomatous structure composed of varying quantities of lymphocytes, endothelial cells, eosinophiles, polymorphonuclear, plasma cells, fibroblasts, reticulum and Sternberg-Reed cells. Clinically the great majority of these patients sought admission because of superficial adenopathy, though in a few instances anemia and cachexia were more prominent.

A few of the patients were observed for short periods only, two to six months, and little information can be derived from them. Many others seen for shorter periods have been entirely excluded. The average duration of the follow-up, however, I

* Read at a Symposium on Tumors, held in New York City under the auspices of the American Society of Clinical Pathologists, June 4, 1935.

† In making this report, I am indebted to Dr. F. C. Wood, under whose direction a large number of these cases were treated with radiation and hence followed for long periods.

TABLE 1

SEVENTY-FOUR CASES OF HODGKIN'S DISEASE

DECADE	CASES	AVERAGE AGE	SEX		INITIAL SYMPTOM	TOTAL DURATION OF DISEASE
			M.	F.		
1st	3	6.5	3	0	Enlarged cervical glands: 3	1 alive to date without symptoms, 8 years. 2 followed for 4½ and 3 years, 7 months respectively
2nd	5	16.2	4	1	Enlarged cervical glands: 4 Abdominal pain: 1	1 died 1 year after onset. 4 followed 2½ years to 8 years after onset; average 27 months
3rd	21	25.0	12	9	Enlarged cervical glands: 16 Enlarged axillary glands: 3 Enlarged inguinal glands: 1 Epigastric fulness: 1	7 died from 2 months to 12 years, 5 months after onset; average duration 23 months. 15 followed from 2 months to 8 years; latter well 1 year ago; average 23 months
4th	19	34.0	11	8	Enlarged cervical glands: 14 Enlarged axillary glands: 1 Generalized weakness, fever, loss of weight: 2 Cough, pain in arm: 1 Pain in sacrum; chills, fever: 1	6 died, 1 to 8 years after onset; average 36 months. 3 alive and well 3, 4, and 6 years to date after onset of disease. 10 followed from 4 months to 8 years after onset; average 29 months
5th	14	44.6	9	5	Enlarged cervical glands: 6 Enlarged inguinal gland: 1 Fever, malaise, weight loss: 3 Nausea and intermittent diarrhea: 1 Substernal pain: 1 Abdominal pain: 1 Tingling sensation, fingers: 1	6 died, 1 to 7 years after onset; average 39 months. 1 alive and well to date 9 years after onset. 7 followed 4 months to 9 years after onset; average 37 months
6th	5	52.7	3	2	Enlarged cervical glands: 3 Enlarged inguinal glands: 1 Constant abdominal pain: 1	3 died from 1 year, 2 months to 5 years after onset; average 30 months. 2 followed 8 and 12 months after onset

TABLE 1—*Concluded*

DECADE	CASES	AVERAGE AGE	SEX		INITIAL SYMPTOM	TOTAL DURATION OF DISEASE
			M.	F.		
7th	4	65.5	2	2	Enlarged cervical nodes: 2 Enlarged axillary nodes: 1 Cough, anorexia, fever: 1	2 died, 7 to 9 months after onset; average 8 months. 2 followed 8 months and 2 years after onset
8th	3	72.6	2	1	Enlarged inguinal nodes: 1 General weakness; loss of weight: 1 Pain over posterior thighs and bladder: 1	2 died 1 and 3 years after onset. 1 followed 3 years after onset

believe rather undervaluates the actual life span of the treated cases, as many were symptom-free when last seen. The accompanying chart denotes the sex, clinical symptoms, and total duration, according to decades (see table 1).

Though Hodgkin's disease is reported in several critical surveys as a disease capable of occurring at any age, there is an unanimity of opinion regarding the high incidence in the early decades of life. Bunting[1] considers it a disease preëminently of youth and early adult life, reporting 122 cases, ninety-eight of which occurred before the thirty-fifth year; of these sixty-three, or slightly over 50 per cent, occurred between the ages of fifteen and thirty. He found a small curve peak in both sexes between the fifth and tenth years, with a drop in both sexes in the ensuing five years, followed by a sudden peak in the male sex between the fifteenth and twentieth years, with a very gradual decline toward the thirtieth year. In the female the peak curve occurred between the twenty-fifth and thirieth years. A much greater proportion of the female cases occurred after the thirtieth year (42 to 27 per cent in the male). Simmonds[5] in 147 cases found the greatest incidence of the disease between the third and fourth decades. Ziegler[7] and Fabian[3] reported similar findings. Wallhauser,[6] in an extensive survey of the world literature found the disease occurring most frequently in young adults from eighteen to thirty-eight years of age. The youngest case recorded is that of Priesel and Winkelbauer.[4] A female infant four and a half

months old at death had apparently had the disease since birth. Necropsy revealed numerous metastases in the skull, lung, bones and superficial and retroperitoneal lymph nodes. A superficial cervical node had been removed from the mother in the last two weeks of her pregnancy, and a diagnosis of Hodgkin's disease was made. The oldest patient previously reported with the disease is that of Fazio which occurred in a male of seventy-six years.

The relative proportion of the disease among the sexes is slightly lower in the cases here reported than that given by Wallhauser, who found the disease 2.3 times more common in males. The follow-up was complete in thirty-two, or 43 per cent of this group. Twenty-seven of these died with an average life span of 33 months, a figure which closely parallels that found by Cunningham,[2] Ziegler, Fabian and others in a large series of cases. Five patients were under treatment and relatively symptom-free to date, averaging six years from the onset of the disease. The disease is considered by most authors as being more rapidly fatal in childhood. Bunting has previously noted that both age and sex have an important influence upon the susceptibility of the lymphoid tissue to disease and play a part in the rate of the disease process. I have had too few cases occurring in the first decade of life to be of statistical value. Twenty-six, or 35 per cent of the total number of the cases, occurred between the ages of forty and seventy-seven, with males predominating 1.6 to 1. A careful review of their clinical behavior, progress, and histological picture shows nothing characacteristic to distinguish them from cases occurring at earlier decades. Their main interest appears to be in the field of differential diagnosis for which reason the following case is reported in some detail:

CASE REPORT

The patient, a white male bond-salesman, seventy-seven years of age, was admitted to the medical ward of St. Luke's Hospital on January 12, 1935, complaining of generalized weakness of one year's duration and loss of fifty pounds weight. His appetite was fair, and he had no gastro-intestinal symptoms or symptoms referable to other organs. His past personal history was irrelevant. His father had died at eighty-four years of age and his mother

at seventy-six, both of unknown causes. A sister and brother were alive and well.

There were a few large hard nodes in both axillas and an enlarged, nodular spleen three finger-breadths below the left costal margin. The liver edge was also palpable three finger-breadths below the right costal margin in the midclavicular line. The peripheral vascular tree showed evidence of advanced sclerosis. Immature senile cataracts were present in both eyes. The blood pressure was 105/65. On the day of admission the blood showed the following: hemoglobin 36 per cent, erythrocytes 2,650,000, leukocytes 20,000, with 80 per cent polynuclears and 20 per cent small lymphocytes. The blood urea was 13.2 mgm. per 100 cc., sugar 110 mgm., uric acid 3 mgm; the icteric index was 8; the Wassermann reaction was negative; the fragility, coagulation and bleeding times were within normal range. Gastric analysis showed 20 per cent free hydrochloric acid, total acidity 39; blood cultures were sterile. X-rays of the long bones, barium clysma, and urine and stool analyses revealed no abnormalities. X-ray of the chest showed fluid in the left chest, and 500 cc. of clear fluid were removed on two occasions. On January 18th the leukocyte count was 15,900 with 94 per cent polynuclears and 6 per cent myeloblasts. During the first week of hospitalization the temperature ranged between 99° and 103°, with daily encursions of 99° to 101° thereafter. Because of the presence of enlarged lymph nodes, a palpable spleen, secondary anemia, and immature leukocytes in the peripheral blood, a diagnosis of an aleukemic phase of myeloid leukemia was suggested. Death took place on February 1, 1935, three weeks after admission. A necropsy was performed fifteen and a half hours after death, the protocol of which follows:

The body is that of a well developed, fairly well nourished white male of seventy-seven years, measuring 176 cm. in length. There is evidence of considerable loss of weight over the thorax and abdomen. Both legs show moderate pitting edema. Decubitus ulcers are present over the upper sacrum and skin covering the left greater trochanter. There is a small abrasion on the dorsum of the right hand. One large discrete egg-shaped node, 5 cm. in diameter, is located on the right axilla, surrounded by several smaller ones. A few large nodes are present in the left axilla. On section the largest node is found to be hard in consistency, and well encapsulated; the cut surface shows large irregular opaque yellowish white areas of necrosis which replace all lymphoid markings. The sclerae have a slight icteric tinge. The pupils are dilated and equal. Bilateral arcus senilis is present. The jaws are edentulous. Rigor mortis is well developed. There is post-mortem lividity of the dependent parts.

On opening the thorax 1500 cc. of slightly turbid greenish fluid is present in the left pleural cavity and 500 cc. of similar fluid in the right. There are no enlarged nodes at the bifurcation of the trachea or at the lung roots. Both lungs show apical pleural scars. The right lung weighs 675 grams. No adhesions are present over its pleural surfaces. On section all the lobes have a congested, slightly edematous, soft cut surface, without evidence of any pneu-

monic or tumor infiltration. The left lung weighs 275 grams, is atelectatic; its pleural surface is smooth. On section it is moderately congested and soft throughout.

The heart weighs 400 grams. A hard yellow white granulomatous node 1.5 cm. in diameter is located in the pericardial sac close to the root of the aorta. There is no excess pericardial fluid. On section the valves appear competent and show no remarkable changes. The coronary vessels are sclerotic, tortuous, and show areas of calcification without any actual occlusion. Sections through the anterior wall of the left ventricle extending out to its apex show a rough fibrotic scar 5 cm. in diameter. On section the myocardium is entirely replaced in this area by dense white fibrous tissue. There are moderate atherosclerotic changes with scarring and calcification throughout the thoracic aorta. These become more marked and numerous throughout the abdominal aorta. A few discrete small hard nodes similar to those found in the pericardial sac are present in the posterior mediastinum. Posterior to the abdominal aorta, just above its bifurcation, are numerous large discrete yellow elastic nodes which on section have a dull yellowish fibrous appearance with replacement of all the lymphoid structure.

On opening the abdomen there is no free fluid in the peritoneal cavity. The liver weighs 1400 grams. Its capsule is smooth, except for one small round nodule 1 cm. in diameter which is present beneath the capsule on the anterior surface of the right lobe. On section the liver tissue appears somewhat atrophic and deeply congested. A smooth-lined angiomatous cyst 3 cm. in diameter is present on the anterior portion of the right lobe. No other remarkable changes are present. The gall bladder appears normal; the extrahepatic ducts are patent. Many large yellowish firm nodes are present at the hilus of the liver and in the gastrohepatic omentum.

The spleen weighs 775 grams. Its capsule is smooth and roughly nodular throughout. On section the organ is firm and studded throughout with elastic opaque yellowish white irregular suet-like areas outlined against a red background giving it the appearance of porphyry. Several large hard nodes are present at the hilus of the spleen and the superior border of the pancreas. The pancreas itself shows no gross abnormalities.

Both adrenals show some thinning of their medullas. The kidneys weigh 100 grams each. On stripping their capsules they have a finely granular red congested surface. Serial sections show fine scars throughout the cortex and medullae; the latter are retracted. The pelves are filled with considerable fat. The smaller blood vessels are patent; their walls appear thickened. The ureters and bladder show no remarkable changes.

There is slight hypertrophy of the median lobe of the prostate, which projects through the internal urethral orifice. Both lateral lobes show moderate adenomatous hypertrophy.

The esophagus appears normal. In the fundus of the stomach beneath the mucosal coat are three round firm nodes 1 cm. in diameter, which have a

yellowish white fibrotic cut surface. A Meckel's diverticulum 4 cm. in length is present about three feet from the ileocecal valve. The colon shows no remarkable changes.

Anatomic diagnosis: Hodgkin's disease. Lymphogranulomatosis of spleen, retroperitoneal and anterior and posterior mediastinal lymph nodes, and liver. Healed myocardial infarct, left ventricle. Bilateral hydrothorax. Nodular endarteritis of aorta. Meckel's diverticulum.

Microscopic examination

Spleen: Sections show the normal architecture of the spleen replaced by a granulomatous type of lesion. The reticulum is greatly thickened and fibrous (fig. 1). In many areas it is hyalinized. Collections of reticulum cells, lymphocytes, polynuclears and fibroblasts are diffusely scattered throughout. Interspersed within these are large cells with polymorphous hyperchromatic nuclei of the Sternberg type. Scattered hemosiderin pigment granules are present. The central arteries show an obliterating type of endarteritis and have lost their adventitial mantle of lymphocytes. A few show granulomatous invasion of their muscle walls (fig. 2). Areas of necrosis are numerous. Eosinophiles are rare.

The abdominal, posterior mediastinal, and axillary lymph nodes show essentially the same structure, the latter showing very large areas of necrosis.

Liver: A few small areas beneath the capsule show the structure of a Hodgkin's granuloma (fig. 3). The surrounding tissue shows some necrosis of liver cells.

Stomach: A small completely hyalinized nodule is present beneath the gastric mucosa, its structure suggesting a fibroma.

Heart: Sections through the left ventricle show an obliterating endarteritis of the small coronary vessels. Considerable edema separates the muscle fibers. A large acellular hyaline scar replaces a portion of the myocardium.

Pancreas: Large irregular areas of acinar necrosis are present. A few of the islets are similarly affected.

Bone marrow from sternum and lumbar vertebrae show no granulomatous infiltration.

Microscopic diagnosis: Lymphogranulomatosis (Hodgkin's type) involving spleen, abdominal, posterior mediastinal, axillary lymph nodes and liver.

Myocardial fibrosis, coronary sclerosis, pancreatic necrosis.

Classifying the seventy-four patients according to age, forty, or 54 per cent, were between twenty and forty, while twenty-six, or 35 per cent, were between forty and seventy-seven. In the former group 75 per cent of the patients complained first of cervical adenopathy. The average life span of those dying of the disease was twenty-nine and one-half months. The latter group

showed 42 per cent complaining of cervical gland enlargement. The life span was twenty-four and one-half months. Both groups showed a moderate secondary anemia on admission, the average having a hemoglobin of 82 per cent with 4,280,000 erythrocytes. No cases showed purpuric manifestations. One

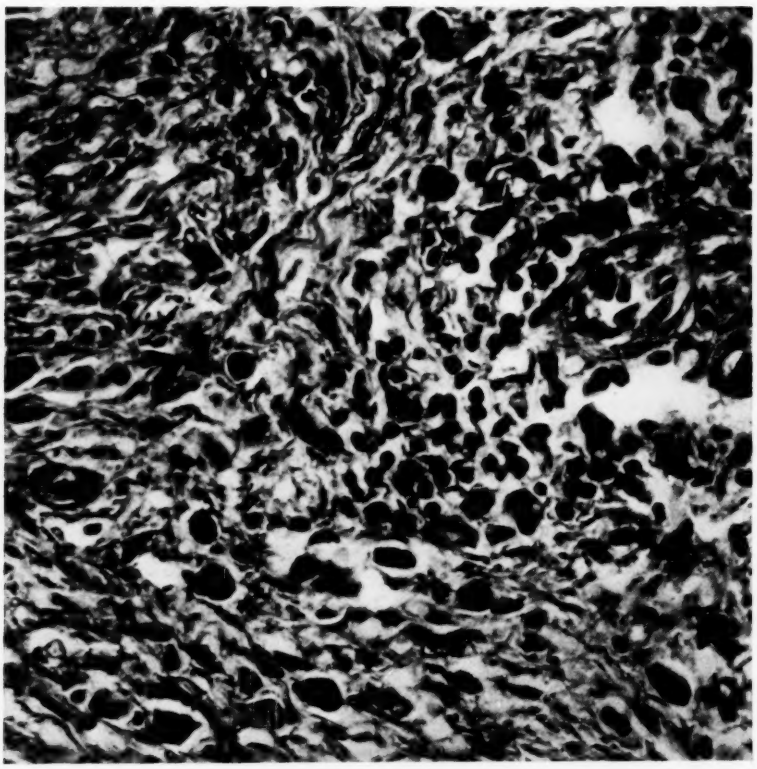

FIG. 1. REPLACEMENT OF SPLENIC PULP BY THICKENED RETICULUM AND POLYMORPHOCELLULAR EXUDATE. × 300

Numerous giant cells of the Sternberg-Reed type are scattered throughout

patient had severe epistaxes which were controlled by transfusion and presented no further hemorrhagic tendencies. The leukocyte counts showed no pathognomonic characters in any age group, but tended to vary more widely than the erythrocyte counts. Slightly over half of the patients had a total leuko-

cyte count within the normal range and an absolute increase in the polymorphonuclear leukocytes. The others showed a moderate leukocytosis with a polynucleosis and a relative lymphopenia. In only two cases was there a suspicion of a leukemic state; a white female twenty-eight years of age had a leukocyte count on

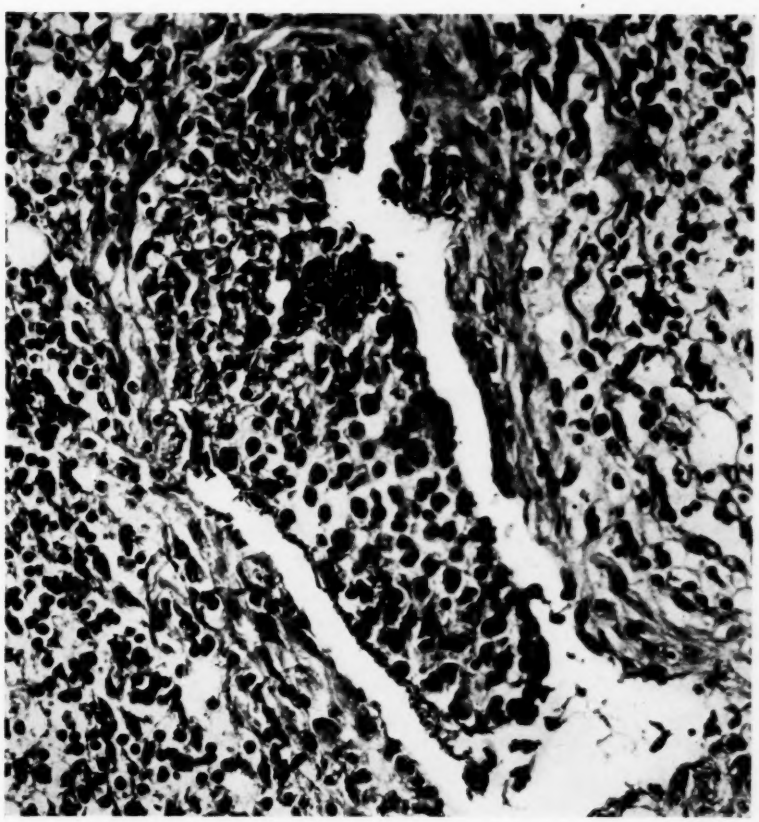

FIG. 2. WALL OF A SPLENIC VEIN INVADED BY GRANULOMATOUS TISSUE. ×300

admission of 45,000 with 94 per cent polynuclear cells and 6 per cent lymphocytes. This remained fairly constant for a month, and then showed 41,400 leukocytes with 72 per cent polymorphonuclear leukocytes, 12 myeloblasts, 4 myelocytes, 1 eosinophile, and 11 small lymphocytes.

In another month, after several x-ray treatments, the total count had fallen to 17,600 with 88 per cent polynuclears and 12 per cent lymphocytes, and thereafter remained at about the same level. The other case was described above.

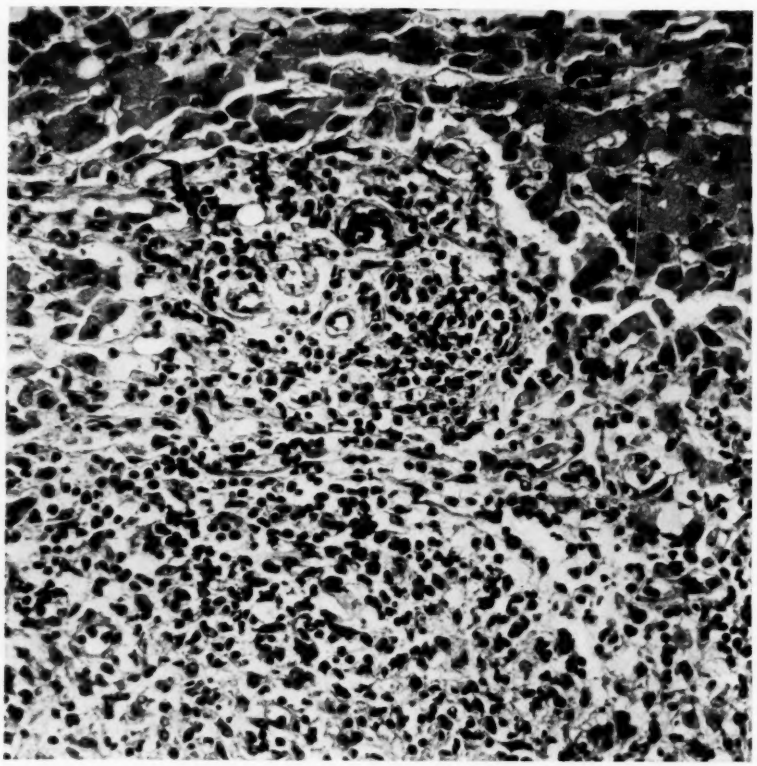

FIG. 3. GRANULOMATOUS TISSUE PENETRATING LIVER PARENCHYMA BENEATH CAPSULE. ×250

Eosinophilia was rarely encountered and apparently bore no relation to the age or type of lesion. The highest count recorded was in a female of thirty-four years of age, with extensive axillary and cervical gland enlargement with pruritus but without involvement of the skin, who had a total leukocyte count of 26,800 with 69 per cent eosinophiles, 29 per cent neutrophilic polynuclear cells, and 2 per cent lymphocytes. On the other hand, the only

patient in this series with very extensive cutaneous granulomatous lesions showed only 6,700 leukocytes with 80 per cent polynuclears and 20 per cent lymphocytes. No clinical or serological evidence of syphilis was noted in any patient. Only one patient had roentgen and clinical evidence of active tuberculosis. Temperature curves were not significant in any age group. The disease was never observed in the negro race. Of ten patients who were eventually examined at necropsy six were forty-five years old and four below 40 years of age. The anatomical distribution of the lesions and their microscopic structure showed the same variation in both groups, regardless of age.

Of the four cases under forty years of age, all showed extensive involvement of the retroperitoneal nodes. Of these three had associated lesions in the lungs, and two showed both spleen and liver involved.

The remaining six cases all showed retroperitoneal node and splenic involvement. Three had associated lesions in the liver and mediastinal nodes. The lungs were uninvolved. One case showed invasion of the lumbar vertebras and another the sphenoid sinus and pituitary gland.

Microscopically the lesions presented essentially the same granulomatous structure in both groups with nothing characteristic in the arrangement and amount of cellular or fibrous tissue.

SUMMARY

(1) A case of Hodgkin's disease in a white male of 77 years is presented with necropsy findings.

(2) A series of seventy-four cases, all microscopically proved, with necropsy findings in ten, are summarized.

(3) Of the seventy-four cases twenty-six, or 35 per cent, occurred in patients ranging from forty to seventy-seven years of age, and forty, or 54 per cent, in patients from twenty to forty years of age.

(4) There is nothing characteristic in the onset, clinical progress and duration of the disease in the latter decades to distinguish it from that occurring in earlier age periods. The anatomical dis-

tribution and histology of the lesion are essentially the same in both groups.

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THE EXPERIMENTAL BACKGROUND AND THE CLINICAL APPLICATION OF THE ESCHERICHIA COLI AND GUM TRAGACANTH MIXTURE (COLI-BACTRAGEN) IN PREVENTION OF PERITONITIS*

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In the earlier reports[7, 10] on the subject of prevention of peritonitis, it was shown that peritoneal protection could be achieved in animals. The earlier methods employed were not, however, practical for use on human beings. In later articles,[8, 11] a material suitable for use in patients was described. The present communication offers: a modification of the material; the clinical experiences in 391 cases accumulated in the hospitals of the city of Toledo†; and the experimental background which led to the development of the method. The material for prevention of peritonitis will be referred to as Coli-Bactragen.

CLINICAL EXPERIENCE WITH COLI-BACTRAGEN

In a recent publication, Potter and Coller,[5] reported the use of Coli-Bactragen for intraperitoneal protection in seventy-nine patients before submitting them to operations upon the colon. They stated that post-operative fatal peritonitis is probably more common in all varieties of surgery of the colon than in any other type of abdominal operation and is an important factor in the high mortality rate. Only one death (on the eleventh post-operative day) in the group of seventy-nine patients, was ascribed to peritonitis. The patient had a combined abdomino-

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† I am indebted to many of the surgeons and especially to Drs. L. F. Smead, F. M. Douglass and W. A. Neill, for their coöperation in the clinical trial of Coli-Bactragen and their kindness in making their records available for study.

perineal resection and, because of failure to heal there was an opening from the perineum into the peritoneal cavity. However, it is likely that the fibrinopurulent peritonitis seen at necropsy was the residual response to the injection of Coli-Bactragen and the individual died of some other cause. The authors observed in their patients at operation, a peritoneal reaction varying from a hyperemia to an abundant exudate resembling, in some instances, a true fibrinopurulent peritonitis. The systemic reactions of their patients consisted of abdominal pain and distention, increase in temperature, pulse rate and peripheral leukocyte count. These

TABLE 1

CLINICAL CASES PROTECTED WITH COLI-BACTRAGEN*

PATIENTS PROTECTED	CLINICAL CONDITIONS IN WHICH COLI-BACTRAGEN WAS USED	DEATHS ATTRIBUTABLE TO CAUSES OTHER THAN PERITONITIS
391	1. Carcinoma of large bowel	Pneumonia
	2. Diverticulitis	Uremia
	3. Intestinal obstruction	Lung abscess
	4. Pelvic inflammatory disease	Lung abscess with empyema
	5. Appendicial abscess	Coronary occlusion
	6. Gastric ulcer	
	7. Appendicitis	
	8. Carcinoma of stomach	
	9. Cholecystitis	

* None of these patients died of peritonitis.

reactions appeared within two or three hours after the introduction of Coli-Bactragen.

Coli-Bactragen was introduced intraperitoneally 12 to 96 hours prior to the operations in 391 patients (table 1). In some instances the condition requiring surgical intervention held little danger of peritonitis, but protection was established to determine the reactions to the protective procedure under all possible circumstances. However, there was a probable mortality of ten per cent due to peritonitis in most of the patients submitting to operation. In none of the patients did peritonitis develop nor was death in those that succumbed ascribed to peritoneal infection. No claim is made, however, that absence of peritonitis in the 391 patients is indicative of absolute efficacy.

The clinical results may be said to be suggestive of a certain degree of peritoneal protection bestowed by the material. It is hazardous to evaluate the efficacy of such a preventive therapeutic measure by statistical studies of a few hundred patients. There are too many variable factors such as the general condition of the individual, the degree of involvement by disease, and the skill of the surgeon which may influence the mortality percentage. Perhaps a better index of the protective value are fourteen persons previously protected with Coli-Bactragen in whom peritonitis was considered as probably inevitable because of the circumstances in the operative procedure. Either feces or pus containing bacteria escaped in large quantities into the peritoneal cavity, but the patients survived without the presence of a clinically demonstrable peritonitis (see table 2).

Coli-Bactragen was introduced in 30 cc. quantities intraperitoneally in the midline of the abdomen, a little below the umbilicus, after the urinary bladder was evacuated. The material was injected from 12 to 96 hours prior to the operation in all but sixteen instances in which it was poured into the peritoneal cavity at the completion of the operative procedure.*

The peripheral leukocyte counts showed a continuous rise reaching the peak in from 24 to 36 hours. The average rise was a little over ten thousand leukocytes. The neutrophilic polymorphonuclears constituted from 90 to 98 per cent of the total number of cells and in the first 16 hours were of the mature type. In the following period, young forms appeared in the average proportion of 6 per cent. Smears and counts of the peritoneal exudate taken at operation, showed a variation in the number of cells from 73,000 to 210,000 per cubic millimeter. The neutrophilic polymorphonuclears composed 98 per cent of the total number and at the end of 72 hours they still constituted 96 to 98 per cent. Young forms were found to vary from 6 to 11 per cent in 24 hours with a slight increase in their number in 48 and 72 hours.

* Coli-Bactragen should not be introduced in the presence of a generalized peritonitis.

The appearance of the peritoneum varied from a moderate injection to an extensive fibrinopurulent exudate covering the peritoneal surfaces with a thick, gray fluid filling the pelvic cavity and the flanks. The more pronounced the peritoneal reaction, the greater were the number of peritoneal cells and the higher the peripheral count. There was no apparent correlation between the intensity of the peritoneal picture and the age, sex or character of disease. In the 391 patients whose records are analyzed here, the convalescence and recovery was uneventful. Of the

TABLE 2

PATIENTS PROTECTED WITH COLI-BACTRAGEN

Because of circumstances in the operative procedure, peritonitis was assumed to be inevitable

PATIENTS	CLINICAL CONDITIONS EXEMPLIFIED	CIRCUMSTANCES CONTRIBUTING TO PERITONITIS	OUTCOME
3	Diverticulum of sigmoid	Feces or pus escaped into peritoneal cavity	Survived. No ill effects. No peritonitis
5	Appendicial abscess	Oozing of pus or feces from abscess into peritoneal cavity at operation	Survived. No ill effects. No general peritonitis after operation. One case with empyema
2	Tubo-ovarian abscess	Pus spilled into peritoneal cavity. Pus contained streptococci	Survived. No ill effects. No peritonitis
4	Carcinoma of colon	Fecal material escaped into peritoneal cavity. Bowel obstructed and thin	Survived. No ill effects. No peritonitis

many more patients outside of Toledo, to whom Coli-Bactragen was administered and whose complete records at the present time are not available for study, there were three instances with morbid changes which may have been caused by the protective material. One patient (in a southern hospital) developed phlebitis; two other patients (in a midwest hospital) some two weeks after injection of Coli-Bactragen and operative procedure, developed ascites and later died. Whether these occurrences were incidental to the disease or operation or were actually induced by Coli-Bactragen is difficult to evaluate, but these cases

are presented as a matter of record. The possibility of formation of peritoneal adhesions due to the presence of the exudate in the peritoneal cavity is a pertinent question. Clinically, it can be answered by prolonged observation of a large number of patients and by the examination of the peritoneum at subsequent operations on the same individuals. In two patients who were reoperated six weeks and two months respectively after the first

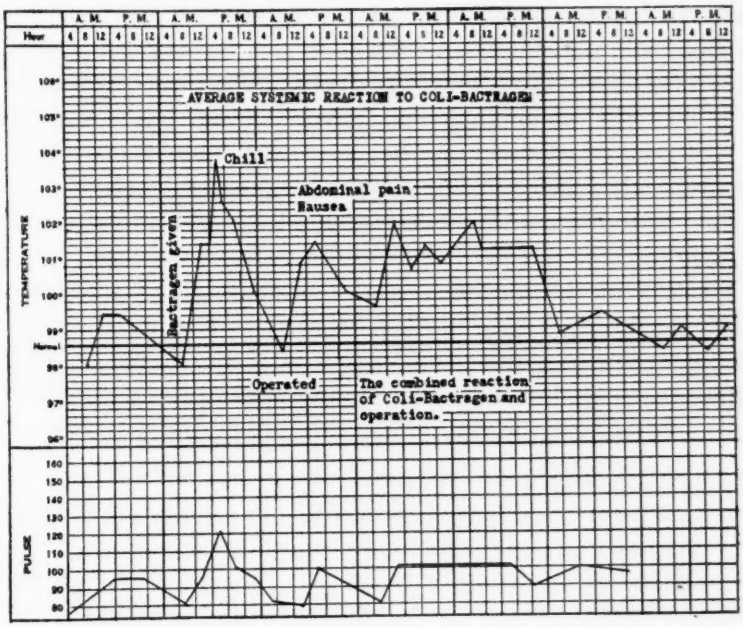

CHART 1

operation at which they had Coli-Bactragen, no adhesions were found. Forty-six patients were followed up for a period of three years and no clinical manifestations of the presence of adhesions were observed. As a matter of fact, preliminary experimental attempts in dogs indicated that 1 to 4 per cent suspensions of gum tragacanth (which forms the vehicle for the Coli-Bactragen) prevents to a great extent, formation of peritoneal adhesions.

Abdominal pain and distention followed by nausea and occa-

sionally vomiting appeared approximately two and one-half hours after the peritoneal injection. The temperature usually rose four degrees with a corresponding increase in the pulse rate. At the height of fever, the patient had a chill (see chart 1). These symptoms were occasionally entirely absent, or varied in degree and disappeared in 24 to 48 hours. When Coli-Bactragen was poured in during the operation, the reaction was completely masked by the anaesthetic and the post-operative course did not appear to be adversely influenced.* The cause of the reactions in people was determined accidently. In an attempt to inject a very obese individual before operation, the material was introduced into the abdominal wall. No reaction followed. When the error was realized, another injection, this time intraperitoneally, was made. The patient responded with the usual reactive symptoms. It is apparent that the mere irritating presence of a foreign substance in the peritoneum, and not some toxic action of the ingredients of Coli-Bactragen, is responsible for the reaction. Dr. J. Shelton Horlsey of Richmond, Virginia, in a personal communication, stated that the injection of Coli-Bactragen 5 or 6 days prior to operation produces a greater degree of protection that a one or two day period interval.

THE COMPOSITION AND PREPARATION OF COLI-BACTRAGEN

Gum tragacanth of the ribbon type is reduced in a mortar to a fine, granular form. The powdered gum tragacanth does not make a suspension of sufficiently large particles and of a desirable consistency. The dry gum is placed in a flask. Aleuronat is triturated to a fine powder, free of lumps, and added to the gum tragacanth and further triturated. The following proportions are used in preparing 100 cc. quantities of Coli-Bactragen: To 1.5 grams (1.5 per cent) of the granular gum tragacanth and 0.5 gram of aleuronat (0.5 per cent) is slowly added, 100 cc. of 0.85 per cent sodium chloride solution, frequently agitating the mixture. It is then agitated at room temperature for three hours until a fairly homogeneous, gummy, suspension is obtained. The mixture is then sterilized in the autoclave at 15 pounds pressure for 45 minutes. The third ingredient consists of formaldehyde killed, washed, colon bacilli. The strain used (our identification number *Esch. coli* 300) has been found to cause a marked production of leukocytes. This capacity, however, varies with the

* These reactions were greatly reduced by killing the bacteria with formaldehyde instead of heat.

strain 300 and requires frequent determinations in order to return it to its original state. Only a few strains out of each hundred examined, showed a sufficient degree of leukocytic stimulation. A 24-hour growth of *Esch. coli* from a stock culture is made on veal agar. The bacteria are washed off with saline and exposed to 0.5 per cent of formol for 48 hours with occasional agitation in a mechanical shaker. The bacteria are then washed four times with saline by centrifugalizing for one hour intervals at 2000 revolutions. The final bacterial sediment is suspended in 0.85 per cent saline solution and shaken to break up the bacterial clumps. Glass beads facilitate the process. The bacterial suspension is then added to the gum tragacanth and the aleuronat mixture. Merthiolate (0.1 per cent of 1:1000) is added. The volume of saline used for suspending the bacteria is equivalent to 2 cc. for each 600 million bacteria. The mixture is agitated in a mechanical shaker for half an hour to insure an equal distribution of the bacteria. The material is stored in 30 cc. vials in 25 cc. quantities. To determine the sterility, 2 cc. volumes are withdrawn from several vials and aerobic and anaerobic cultures are made. The cultures must be kept in the incubator for 96 hours. Formaldehyde may inhibit bacterial growth for that period. Approximately 600 million colon bacilli are present in each vial of gum tragacanth and aleuronat. Roughly, the growth on two medium sized slants of agar correspond to that number of bacteria. The vials should be kept in a cold place, preferably a refrigerator. There is no apparent deterioration for a period of a year and a half although, it has been our practice to make up new batches every 6 months. To summarize the composition of Coli-Bactragen in 100 cc. quantities, from which four vials can be made up:

Gum tragacanth (ribbon)	1.5 per cent (1.5 grams)
Aleuronat	0.5 per cent (0.5 gram)
Esch. coli (300)	2400 million
0.85 per cent salt solution	100 cc.

After the Coli-Bactragen is prepared, it is tested for the following properties:

(1) The ability to produce a local peritoneal leukocytosis of a sufficient large number of leukocytes (not less than 70,000 per cubic millimeter of peritoneal exudate) within a few hours (3 to 6).
(2) The ability to stimulate the bone marrow to further production and expulsion of leukocytes.
(3) The absence of substances which interfere or inhibit (usually a soluble toxic substance of an unsuitable *B. coli* strain) phagocytosis.
(4) The retention of the Coli-Bactragen in the peritoneum.
(5) The absence of injurious local or general effects and the limitation of the reaction to the usual type (see chart 1).

The presence of peritoneal leukocytosis is determined by the intraperitoneal introduction of Coli-Bactragen into three dogs and puncturing the abdominal wall at half hour intervals with a glass capillary pipette. The fluid thus ob-

tained, is taken up into an erythrocyte and leukocyte counting pipette and leukocyte counts are made. Smears are stained with Wright's stain for determination of type of cell. Neutral red and Janus green supravital preparations are studied whenever Wright's stain is insufficient for cell type differentiation. Twenty-four hours after introduction of Coli-Bactragen two slants of viable and virulent *Esch. coli* suspended in 2 per cent gum tragacanth are introduced into the peritoneal cavity and hourly abdominal punctures are done for six consecutive hours and at 12 hour intervals for 3 days. The survival of at least two of the three dogs (average weight of 20 kilos) indicates the efficacy of the preparation. Peritoneal cell counts and smears show the degree of the leukocytic response and the presence of satisfactory phagocytosis. It has been shown[6] that the presence of a toxin in the peritoneal cavity interferes with phagocytosis of the invading bacteria. Diphtheria toxin was introduced into the peritoneal cavity of an immunized animal with peritonitis. Ordinarily, such an animal disposed of the bacteria of the peritoneum within a few hours by means of a rapid phagocytosis. The addition of the toxin, however, resulted in the retention of a large number of free bacteria. The polymorphonuclears were found to be either free or with very few ingested bacteria. It appeared that the probable cause of this interference with phagocytosis was due to some action on the polymorphonuclears by the toxin. The bone marrow reaction is indicated by appearance of young cells in the peritoneal exudate. The temperature and the observation of the animal during the 24 hours with Coli-Bactragen alone presents the general reaction of the animal to the material.

THE EXPERIMENTAL BACKGROUND

Goldblatt and I[4] injected killed *Esch. coli* suspended in saline intraperitoneally or subcutaneously into dogs. Four injections were given with intervals of three days between each. Ten days after the last immunizing dose, peritonitis due to colon bacilli was induced. The peritoneally and subcutaneously immunized dogs survived while an equal number of controls died. It was assumed that an immunity which at least in part was general in type, against peritonitis due to colon bacilli could be produced. As the interval between the last immunizing dose and the onset of peritonitis was reduced, the percentage survival decreased. The best results (all surviving) were achieved 10 to 14 days after the last injection of the vaccine (see table 3). These findings further emphasized the general nature of the immunity obtained. The number of vaccinations were then varied. A single vaccination resulted only in 11.7 per cent survivals. The highest percentage of survival obtained with one, two or three successive vaccina-

tions did not exceed twenty per cent while four vaccinations resulted in 64.2 per cent (table 4). These experiments suggested that enough antigen must be introduced, that is, a sufficient degree of immunity must be established as well as sufficient length of time must elapse before satisfactory results in survival from peritonitis due to *Esch. coli* can be achieved.

TABLE 3

PROTECTION OF DOGS AGAINST COLON BACILLUS PERITONITIS FOLLOWING PERITONEAL VACCINATION WITH A SALINE SUSPENSION OF ESCH. COLI ON FOUR SUCCESSIVE DAYS

The peritonitis was produced from 1 to 24 days after the last vaccination

DAYS BETWEEN LAST VACCINATION AND PERITONITIS	DOGS USED	DOGS SURVIVING	SURVIVAL
			per cent
1	28	18	64.2
7	9	6	66.6
11	5	5	100
14	9	9	100
24	10	8	80

TABLE 4

PROTECTION OF DOGS AGAINST COLON BACILLUS PERITONITIS 24 HOURS AFTER VACCINATION WITH A SALINE SUSPENSION OF ESCH. COLI

The number of vaccinations varied from 1 to 4 administered on successive days

VACCINATIONS	DOGS USED	DOGS SURVIVING	SURVIVAL
			per cent
1	17	2	11.7
2	10	2	20.0
3	10	1	10.0
4	28	18	64.2

An attempt was made to determine whether immunization with *Esch. coli* suspended in saline would be of value in fecal peritonitis.[10] (Feces contained several species of bacteria incident to the intestinal tract.) Four successive injections of *Esch. coli* (living bacteria in one group and heated bacteria in another group of dogs) were made intraperitoneally and on the 15th day after the last vaccination, 5 grams of feces suspended in

saline were injected into the peritoneal cavity of the dogs. Ten out of eleven animals (over 90 per cent) vaccinated with living bacteria survived while only three out of eight (over 36 per cent) vaccinated with heat killed bacteria remained alive. Out of fifteen control dogs with a similarly induced fecal peritonitis all died. It was suggested at that time that the survival of the animals with fecal peritonitis may be due either to a production of nonspecific antibodies (humoral, cellular or both) or that the colon bacillus in the feces was the prime cause of the peritonitis and death. Consequently, immunization with colon bacillus resulted in a specific immunity against the organism.

If the immunity obtained was a result of a production of specific antibodies, vaccination with several bacteria (which are commonly present in the intestinal tract) would be apt to be more efficacious than vaccination with a single bacterial organism. A vaccine consisting of the *Esch. coli* (not strain 300), *Streptococcus fecalis*, and *Clostridium welchii* was made up in saline and two groups of dogs were vaccinated four times on successive days. In one group (twenty-one dogs) peritonitis was induced the day after the last vaccinating dose while the other group (ten dogs) was given peritonitis 24 days later. Along with the animals immunized with the bacterial mixture, two other groups were vaccinated with a saline suspension of *Esch. coli* 300. Of the twenty-one animals with the bacterial mixture and peritonitis a day after the last dose, only five survived (23.8 per cent) while of the twenty-eight dogs vaccinated with *Esch. coli* eighteen survived (64.2 per cent). In the group with peritonitis 24 days later, out of ten dogs with bacterial mixture, six survived (60 per cent) and the percentage survival of the *Esch. coli.* 300 immunized dogs was eighty (see table 5). Apparently, as far as these experiments indicated, vaccination with multiple organisms resulted in a lesser degree of immunity than the employment of *Esch. coli* alone. These experiments suggested that the reason for the immunity probably did not lie in the production of specific antibodies.

Further experiments were attempted to establish the nature of the immunity.[13] Dogs were immunized with *Esch. coli* sus-

pended in saline and peritonitis induced by injection of either colon bacilli or fecal material containing many species of bacteria. At ten minutes and later at hourly intervals peripheral blood studies were performed and peritoneal exudate was obtained by abdominal punctures with a glass pipette. The total number of leukocytes and number of bacteria were counted. Differential counts were made by staining with Wright's stain and supravital dyes (neutral red and Janus green). At intervals of one to 6 hours for 96 hours a dog was killed and sections were taken of the peritoneum, omentum, mesentery, diaphragm, intestines, lymph nodes, spleen and kidneys. The tissue was fixed in neutral

TABLE 5

PROTECTION OF DOGS AGAINST PERITONITIS FOLLOWING FOUR DAILY SUCCESSIVE PERITONEAL VACCINATIONS OF A BACTERIAL MIXTURE

The mixture *suspended in saline* included *Esch. coli* (not *Esch. coli* 300), *St. fecalis* and *Cl. welchii*

VACCINATING MATERIAL	DAYS BETWEEN LAST VACCINATION AND PERITONITIS	DOGS USED	DOGS SURVIVING	SURVIVAL
				per cent
Bacterial mixture	1	21	5	23.8
Esch. coli 300 vaccine	1	28*	18	64.2
Bacterial mixture	24	10	6	60.0
Esch. coli 300 vaccine	24	10	8	80.0

* Same group as in tables 3 and 4.

Zenker-formaldehyde and stained with hematoxylin-eosin and Gram's stains. Thus, during the course of peritonitis, the peripheral blood, the peritoneal exudate and the tissue reactions were correlated.

Only those phases of the experiment pertinent to the determination of the nature of the immune process will be presented. The peritoneal exudate of the immunized animals showed a large number of leukocytes prior to the induction of peritonitis. These leukocytes dropped consistently in number during the first two hours in the course of peritonitis and showed a more or less regular fluctuation for 4 days. These fluctuations almost formed a

pattern. It was observed that those animals which had a peritoneal leukocyte count, prior to the onset of peritonitis, of less than 36,000 per cu. mm., although vaccinated, did not survive. The successfully vaccinated dogs throughout the course of peritonitis had a consistently high number of leukocytes. The control animals, not vaccinated, had uniformly a low number of peritoneal leukocytes. The leukocytes were predominantly poly-

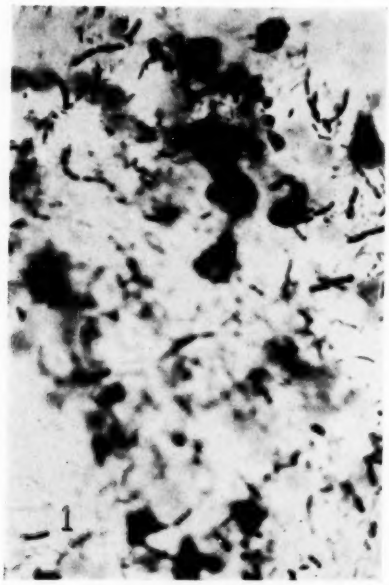

FIG. 1. SMEAR OF THE PERITONEAL EXUDATE 1 HOUR AFTER ONSET OF PERITONITIS IN A CONTROL, NON-VACCINATED DOG

There is a very large number of free bacteria and some polymorphonuclears with phagocytosed organisms.

morphonuclear in type (98 to 100 per cent in the first 48 hours and 88 to 92 per cent in the following 48 hours). As will be pointed out later, the decisive period in which the infection overcomes the animal is determined in the first 5 to 8 hours in the course of the peritonitis. Hence, it is apparent that whatever function the leukocytes bear in relation to the infection, that function is performed by the polymorphonuclear cells. The

type of leukocytes in the peritoneal exudate of the vaccinated animal (11 days after the initial vaccination) prior to the onset of peritonitis, was also predominantly polymorphonuclear.

The bacterial counts of the peritoneal exudate in the vaccinated dogs showed a rapid decline from the second to the fourth hour. Although viable bacteria were found in small, though successively diminishing numbers for 7 to 11 days after the onset of peritonitis,

FIG. 2. SMEAR OF THE PERITONEAL EXUDATE 1 HOUR AFTER ONSET OF PERITONITIS IN A VACCINATED DOG

There are no free bacteria in this field but some of the polymorphonuclears show ingested organisms.

no free microörganisms were seen in the smears. The viable bacteria were phagocytosed and were derived from the polymorphonuclears which were broken down in the cultural procedure. The control, non-vaccinated animals, revealed a rapid rise in the bacterial counts in from the second to the third hour which persisted with a slight decline to the time of death.

Sections of the tissues made during the course of peritonitis

of the vaccinated dogs, showed presence of polymorphonuclears in the lumina of capillaries and arteries within 15 minutes. In one hour, the vessels were crowded with these leukocytes, many of which already appeared in the surrounding tissue. In some of the vessels, the leukocytes congregated at the inner wall with many polymorphonuclears within, or just outside the vessel. In succeeding hours, the leukocytes greatly increased in number.

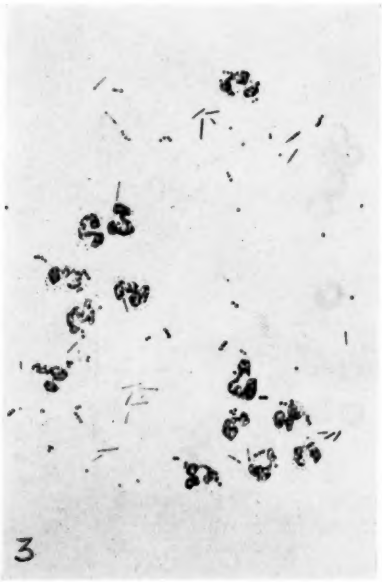

FIG. 3. SMEAR OF THE PERITONEAL EXUDATE 8 HOURS AFTER ONSET OF PERITONITIS IN A CONTROL, NON-VACCINATED DOG

There are numerous free bacteria and many of the polymorphonuclears show various stages of disintegration.

In 96 hours, the predominant cell was still the poymorphonuclear (see figures 6 and 8). In the control, non-vaccinated animals, within the first hour, the capillaries and the arteries became dilated and were filled with blood and a small sprinkling of polymorphonuclears within the vessels and outside in the tissues. Succeeding hours up to the time of death showed but little variation except for a more intense engorgement of the vessels with

erythrocytes and a still greater diminution in the number of leukocytes (see figures 5 and 7).

These experiments indicate that immunization with colon bacilli (300) suspended in saline, results in a local peritoneal leukocytosis of the polymorphonuclear type and that the leukocytes phagocytose the invading bacteria irrespective of the species. The phagocytosis is complete in 4 to 8 hours. The

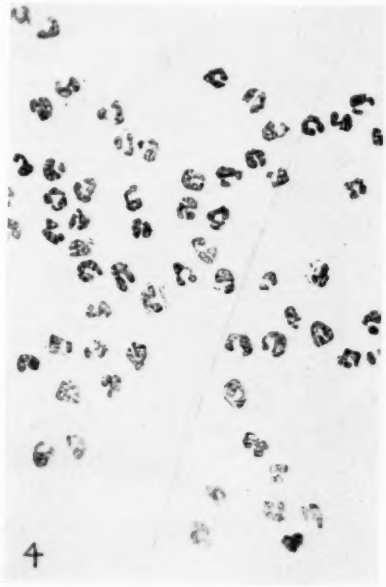

FIG. 4. SMEAR OF THE PERITONEAL EXUDATE 8 HOURS AFTER ONSET OF PERITONITIS IN A VACCINATED DOG

There are no free bacteria. Polymorphonuclears are numerous and intact for the greater part. There are several cells with phagocytosed bacteria.

leukocytosis is a local manifestation of a general mobilization of the cells with an increase in the production and expulsion in the bone marrow. The presence of a large number of polymorphonuclears in the peritoneal exudate, the disappearance of the free bacteria, the observation of leukocytes with ingested bacteria, the marked polymorphonuclear tissue reaction in the vaccinated animals, and on the other hand, a relatively small number of

leukocytes, the progressive increase in the bacterial population and the paucity of tissue leukocytosis in the control dogs, form the basis for these conclusions. Since the control animals died in 8 hours and the vaccinated ones showed a complete disappearance of free bacteria in the peritoneum, the assumption is that the result of the infection is determined within that time. Since the leukocytes evoked by a colon bacillus vaccine, protected the

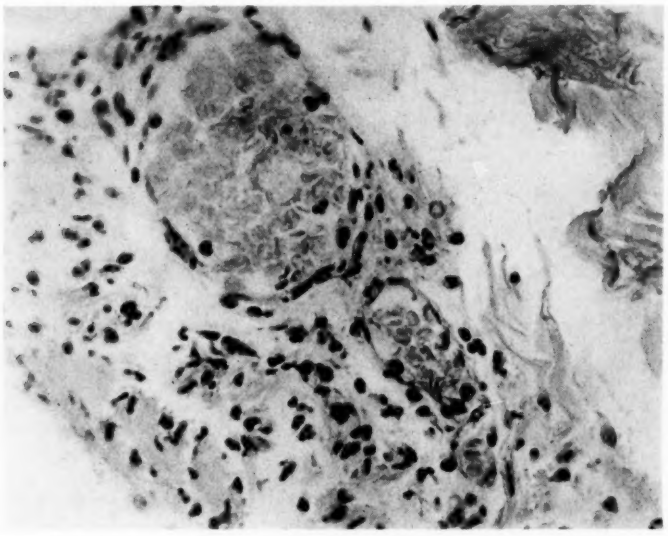

FIG. 5. SECTION OF OMENTUM 1 HOUR AFTER ONSET OF PERITONITIS IN A CONTROL, NON-VACCINATED DOG

The capillaries and arteries are dilated and filled with blood. There is a small number of polymorphonuclears within the vessels and in the surrounding tissue.

animal against a fecal peritonitis it is hardly to be supposed that any specific immunity was present. It may be assumed that a non-specific phagocytosis by the polymorphonuclears was the deciding factor. The experiments reported thus far, however, do not clarify the rôle that the humoral antibodies, evoked by the vaccination, may have had in the protection. As will be described later, further experiments do not show evidence that the humoral antibodies play an important part. On the basis of this

work, the conception of a local immunity in the sense of Gay and his coworkers[1, 2, 3] defined by them as an "acquired, increased protection of some part of tissue superior to that existent elsewhere in the body," is not acceptable as far as dogs and the peritoneal cavity and immunization with colon bacillus is concerned. Furthermore, under the conditions of these experiments, the polymorphonuclears and not the clasmatocytes (i.e., histio-

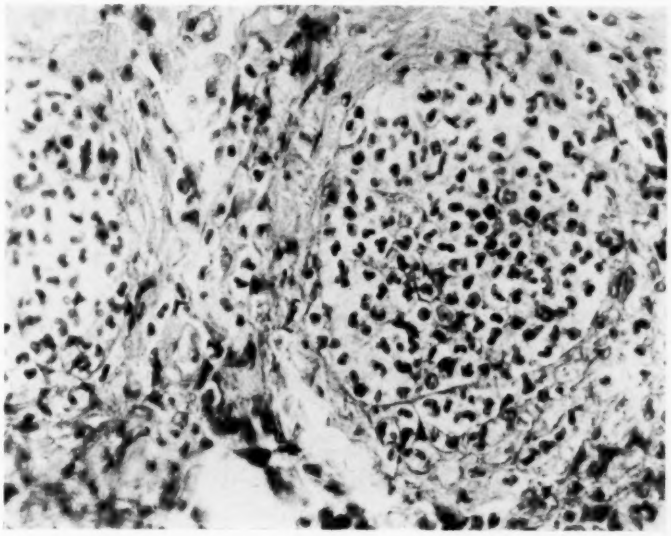

FIG. 6. SECTION OF OMENTUM 1 HOUR AFTER ONSET OF PERITONITIS IN A VACCINATED DOG

The arteries are filled with polymorphonuclears which are also present in the surrounding tissue.

cyte, macrophage) are responsible for bacterial phagocytosis and for the probable resulting protection.

If the peritoneal protection is due to a local accumulation of leukocytes and subsequent phagocytosis, protection against peritoneal infection should be possible to establish in a few hours, providing a sufficient number of leukocytes is brought to the peritoneum. Bacterial antigens suspended in saline, rapidly leave the peritoneal cavity through capillaries and lymphatics

and as a consequence, although the bone marrow activity is stimulated, very few cells migrate to the peritoneum. Repeated injections of saline suspended antigens, produce peritoneal injury and inflammation, and a resulting slowing up of the passage of the vaccine from the peritoneal cavity. If the antigen were suspended in some vehicle which considerably slowed up its

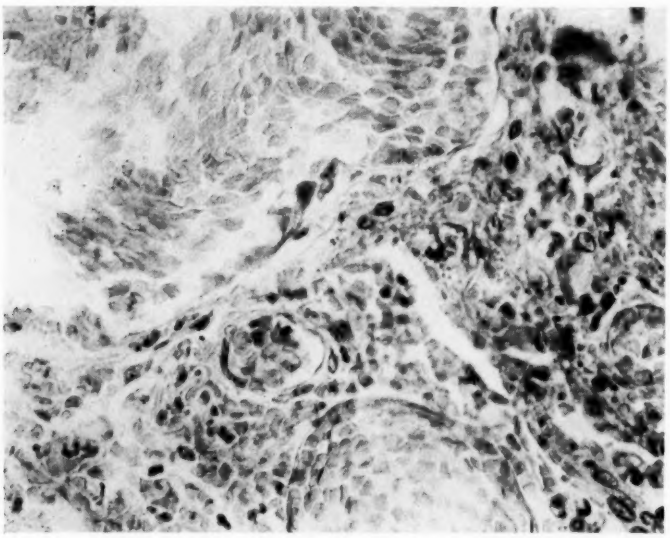

FIG. 7. SECTION OF OMENTUM OF A CONTROL, NON-VACCINATED DOG 8 HOURS AFTER ONSET OF PERITONITIS

The capillaries and arteries are dilated and filled with blood. There is only an occasional leukocyte.

passage, the necessary conditions for a rapid protection would be present.

In previous experiments to determine the cause of death in peritonitis, it was found that when *Esch. coli* were suspended in gum tragacanth and injected intraperitoneally, the bacteria remained almost entirely in the peritoneal cavity.[9] While the thoracic duct lymph contained only 380 bacteria per cc. 21 minutes after the intraperitoneal injection of *Esch. coli* in gum tragacanth, and the blood was sterile, there were 5,240,000

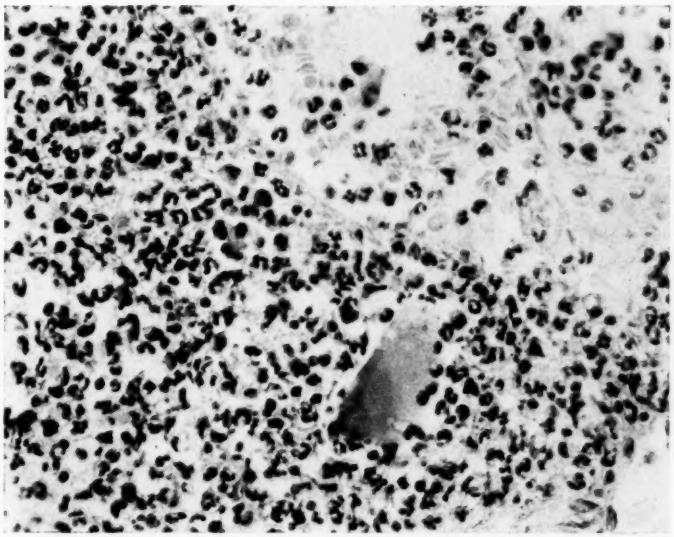

FIG. 8. SECTION OF OMENTUM OF A VACCINATED DOG 8 HOURS AFTER ONSET OF PERITONITIS

The tissue is filled with polymorphonuclears. Contrast the picture with that of the control dog in Figure 6.

TABLE 6

PERITONEAL CELL AND PERIPHERAL BLOOD COUNTS DURING THE PROTECTIVE STAGE IN A DOG WITH COLI-BACTRAGEN

HOUR FOLLOWING INTRODUCTION OF COLI-BACTRAGEN	PERITONEAL COUNT PER CUBIC MILLIMETER OF EXUDATE	PERIPHERAL COUNT (10,600 BEFORE INTRODUCTION OF COLI-BACTRAGEN)
1		12,800
3	91,000	15,600
5	83,300	21,500
12	69,900	32,300
24	76,000	29,700
26	72,100	32,650
30	60,600	22,450
34	60,000	21,400
48	109,600	24,000
50	99,000	20,000
54	138,000	19,300
72	240,000	20,450
96	111,000	20,300
122	244,000	25,600

bacteria per cubic centimeter when *Esch. coli* was suspended in saline.

Escherichia coli (300) was suspended in gum tragacanth and injected intraperitoneally. Leukocytes counts were made from the peripheral blood and the peritoneal exudate (see table 6). There was a rapid appearance of leukocytes of the polymorphonu-

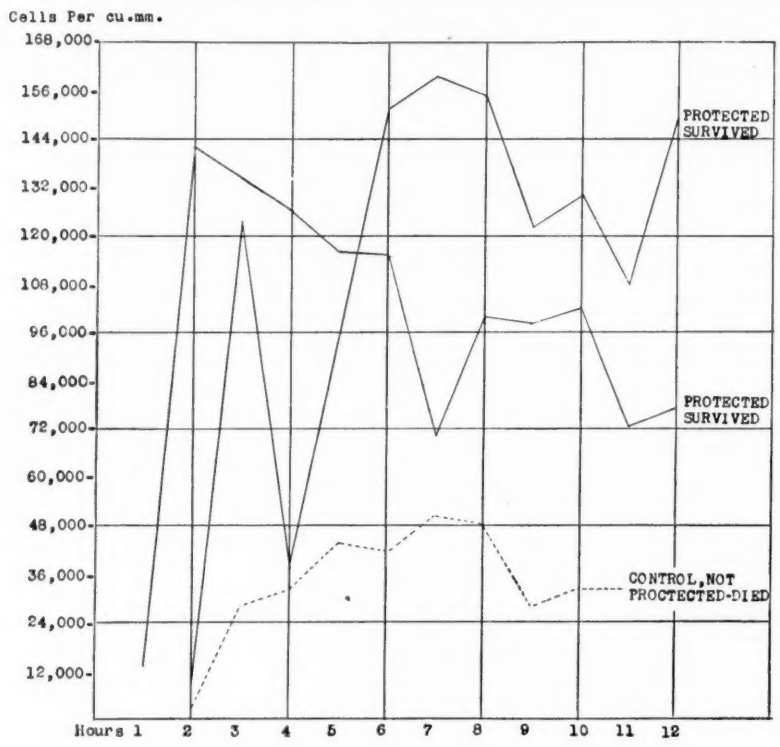

CHART 2. LEUKOCYTE COUNTS OF THE PERITONEAL EXUDATE DURING THE COURSE OF PERITONITIS IN DOGS PROTECTED WITH COLI-BACTRAGEN

clear type in the peritoneum. In 3 hours there were 91,000 cells per cubic millimeter, a greater than necessary number of cells to insure peritoneal protection.

Peritonitis was induced in dogs protected with *Esch. coli* suspended in gum tragacanth. Peritoneal and bacterial counts and smears were made of the exudate at frequent intervals. In the

first hour, the leukocytes already present, were diminished in number. In the second and third hours, the number of cells showed a marked increase and from then on it varied hourly reaching its maximum in the 96 to the 122nd hour (see table 6). Comparative counts in protected and non-protected dogs revealed a quantitative difference in the leukocytes. The unprotected animals consistently showed a low number of leukocytes (see chart 2). The smears of the peritoneal exudate in the protected dogs revealed complete phagocytosis of all bacteria by the end of

TABLE 7

PROTECTION OF DOGS WITH COLI-BACTRAGEN FOLLOWED BY PERITONITIS

TYPE OF PERITONITIS	INTERVAL BETWEEN PROTECTION AND PERITONITIS	DOGS USED	DOGS THAT DIED	SURVIVAL
	hours			*per cent*
Colon bacillus	12	5	3	40
	18	6	0	100
	24	19	3	84
	48	35	7	80
	72	9	2	77
Appendix perforated, feces smeared over peritoneum—appendicial perforation left open	48	6	2	66
Colon bacillus	Control dogs. No protection	40	40	
Appendix perforated, feces smeared over peritoneum—appendicial perforation left open	Control dogs. No protection	10	10	

the sixth hour, although the microörganisms remained viable within the leukocytes for a period of 4 to 11 days as determined by culture and bacterial counts of the leukocytes. The leukocytes throughout the time of the experiment were predominantly of a polymorphonuclear type and at no time less than 90 per cent of the total cell number.

To determine the degree of protection conferred by the gum tragacanth and *Esch. coli* 300 mixture, a number of dogs were injected with this mixture and at varying intervals following it,

peritonitis (colon bacillus or a mixed infection by perforation of the appendix and smearing feces over the peritoneum) was induced. Table 7 illustrates the results obtained. The peritoneal infection was invariably many times the lethal dose necessary to produce death. The potency of the method is shown by the rapid death of the control animals and by the severity of the local lesion produced.* In this particular set of experiments, the highest degree of protection was achieved in 24 hours after protection. In other sets of similar experiments, the greatest percentage survival varied between 3 to 72 hours.

To evaluate the protective capacity of other substances, which at some time have been used for stimulation of leukocytes or for

TABLE 8

ATTEMPTED PROTECTION OF DOGS AGAINST COLON BACILLUS PERITONITIS BY INTRAPERITONEAL INTRODUCTION OF VARIOUS SUBSTANCES

MATERIAL USED FOR ATTEMPTED PROTECTION	DOGS USED	DOGS SURVIVING	SURVIVAL
			per cent
Gum tragacanth alone	15		
Typhoid bacteria in gum tragacanth	4		
Milk with gum tragacanth	4		
Amniotic fluid	4		
Aleuronat alone	4	1	25
Aleuronat in gum tragacanth	10	3	30

peritoneal protection, dogs were injected with the respective materials and 24 hours later, peritonitis due to *Esch. coli* was induced (see table 8). Peritoneal smears and cell and bacterial counts were done on each group. Except for the aleuronat, none of the other substances resulted in survival of any of the animals. The studies of the peritoneal exudates revealed nothing suggestive to justify further investigation of the other substances used. The gum tragacanth, however, did show a higher leukocytic response than any of the other materials except aleuronat; the animals injected with the gum lived longest. Because of the

* Whenever a single control animal survived, the entire experiment was discarded. It was considered essential that the peritonitis should be lethal for all control animals.

peritoneal picture and the survival of 30 per cent of the animals injected with aleuronat and gum tragacanth, aleuronat was added to the original *Esch. coli* and gum mixture.

DISCUSSION

It may be deduced from the above experiments that gum trgacanth successfully retains the bacteria within the peritoneal cavity. This retention results in a local outpouring of a large number of polymorphonuclear leukocytes within three hours following the introduction of the bacteria (*Esch. coli* 300) suspended in gum tragacanth. When viable, virulent bacteria which produce peritonitis were introduced intraperitoneally or the appendix was ruptured and feces smeared over the peritoneum, under the conditions of these experiments, the offending bacteria were rapidly phagocytosed by the polymorphonuclears. It was assumed that the rapid bacterial phagocytosis was responsible for the large percentage of survivals of the animals from an otherwise lethal peritonitis. Because of the rapidly established protection (3 to 12 hours) it is not believed that humoral antibodies play any part except in-so-far as the opsonins in the serum exudate may aid the process of phagocytosis. The difference between a normal, non-protected, and protected animal in withstanding a peritoneal infection is believed to be largely a quantitative leukocytic factor; the protected dog has available and can evoke a larger supply of polymorphonuclears than the control dog. The protection is not limited against a single organism such as *Esch. coli* but is efficacious against other bacteria commonly found in feces. This is evidenced by the phagocytosis of, other organisms than *Esch. coli* and survival of animals with a mixed bacterial infection. The phagocytosis is accomplished rapidly prior to the production of soluble toxic substances, which are assumed to cause abnormal changes in the vascular system and possibly other organs[12] and death.[6]

SUMMARY

(1) A material is presented for protection against peritonitis.
(2) This material was used in a large number of patients. In

391 patients under observation in the hospitals of Toledo, in whom the protective substance was used, there was not a single instance of peritonitis though many of the patients were exposed to the infection.

(3) The composition and the method of preparation of this material is described.

(4) The essential purpose of the material is to allow a very slow passage of leukocyte stimulating substances from the peritoneum and to attract the leukocytes into the peritoneal cavity.

(5) The mechanism which operates in protecting against peritoneal infection is assumed to be one of local leukocytosis and phagocytosis. A sufficiently large number of polymorphonuclears are present in the peritoneal cavity at the onset of infection and leukocytic production and expulsion is enhanced. These leukocytes promptly ingest the bacteria thus preventing the local, reactive, protective, inflammatory process (peritonitis) and the elaboration of soluble toxic substances which are believed to be the cause of death in peritonitis.

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REPORT ON A CASE OF UNUSUAL GIANT CELL LYMPHOGRANULOMA*

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The examination of granulomatous lesions of various types is a common and often puzzling task in the surgical pathological laboratory, often they appear to defy classification on an etiological basis, which is not at all strange when one considers how many years medical science has busied itself with the as yet unsolved problem of the etiology of Hodgkins' lymphogranuloma. Granulomas caused by various higher bacteria and fungi are not uncommon and, recently, there has been added to these another type resulting from infection by an ultramicroscopic virus, the lymphogranuloma inguinale. The following case presents many interesting features and, although one can not be certain as to its etiology at the present moment, it is well to publish it in the hope that similar cases may be observed and reported and, in this way, establish an entity if such a one exists.

CASE HISTORY

The patient was a young German housewife of thirty-four years of age. She came to the Out Patients' Department complaining of what she called "lumps in her skin" which had been appearing and disappearing for the past year, chiefly on the face, arms and buttocks, although other parts of the body were not spared. These nodules were in, or just beneath the skin and first appeared at the center of a diffuse and angrily flushed area which soon faded, leaving elongated lumps that gradually increased in size during about six weeks, became red and raised above the skin level and were elliptical, or bolster-shaped and quite painless. They would then remain stationary in size for about two or three months, when they would gradually pale off and subside, leaving bluish nodules just beneath the surface that ultimately became scar-

* Read at a Symposium on Tumors, held in New York City under the auspices of the American Society of Clinical Pathologists, June 4, 1935.

like, whitish masses. The lesions never ulcerated unless traumatized and the patient wished to have treatment more on account of cosmetic reasons than because she was at all troubled about her condition. She had had deeper nodules in her neck which, on removal at a neighboring hospital, proved to be enlarged lymph nodes and were diagnosed as "atypical tuberculosis." During her course of investigation in the dispensary she developed an inguinal adenitis in which the lesion was in every way similar to that seen in the cervical nodes, sections of which had been kindly submitted to us for examination.

There was nothing of note in her past history that might explain her present condition if one excepts two attacks of what might be considered rheumatic fever; one of these occurred at the age of twenty-one when she had fever, a generalized rash and intense pain in her hands and feet, with swollen ankles and wrists. This kept her in bed for nine weeks, after which the attack subsided rather suddenly. A similar but slighter attack came on about two years ago, with fever, sweating and pain in the right arm. This, too, subsided rather suddenly. In her personal history there was nothing that bears on the case excepting the fact that she had one miscarriage and no pregnancies since then; her Wassermann test was negative.

Physical examination

The salient features of this were the skin lesions and the mass in the left groin. The lesions were as just described: raised, rubbery, elliptical masses with rounded extremities varying in color from bright red to pinkish red and covered with thin, shining skin; older bluish lesions were found here and there beneath the skin and there were many white scars. The scars of several biopsies were also present. The typical lesion was about 3 cm. long, 1 cm. wide and 3 mm. above the surrounding surface; the bluish lesions were much smaller, about 8 mm. in diameter and more or less spherical. There was a slightly indurated zone about the fresh lesions, but this was not accompanied by fixation to the underlying tissue. The mass in the left groin had the appearance and feel of a chronic bubo of some sort. One of the skin lesions, on the left calf, was ulcerated over an area of about 5 mm.; there was a history of trauma in this instance. The lesions were all quite painless and rather rough examination failed to elicit any tenderness.

Course of illness

The inguinal nodes were removed at operation, after which the patient came in from time to time and the treatment of the case was necessarily largely of a supportive nature pending more exact diagnosis. Finally, one of the skin lesions was excised under careful asepsis and ground up in toto, some of the material being cultured on various mediums and the rest injected into a guinea pig. The cultures showed an anaërobic, gram-positive, branching organism with small bulbous conidia at the ends of chains composed of bacillus-like segments. This gradually became aërobic on further cultivation. It was diag-

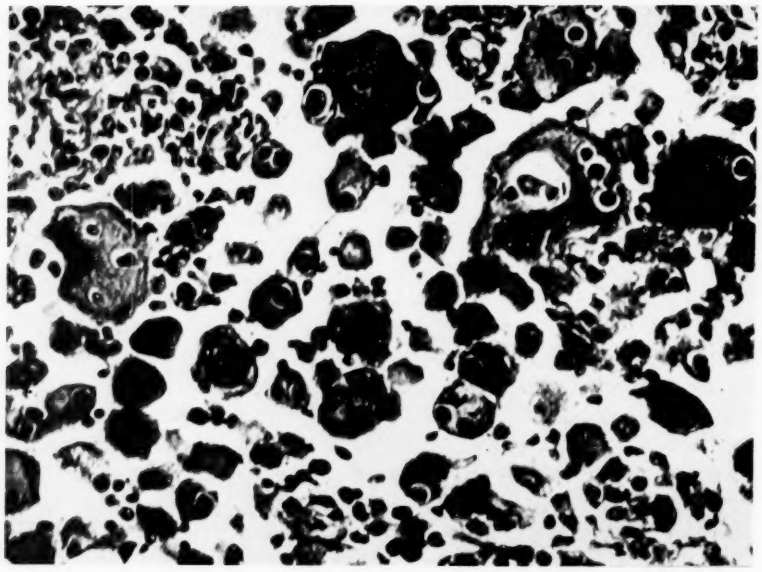

FIG. 1. GIANT CELLS AND OTHER INFLAMMATORY CELLS IN LOOSE ARRANGEMENT AT THE CENTER OF ONE OF THE DEGENERATED LESIONS
The nuclei are very pyknotic. ×220

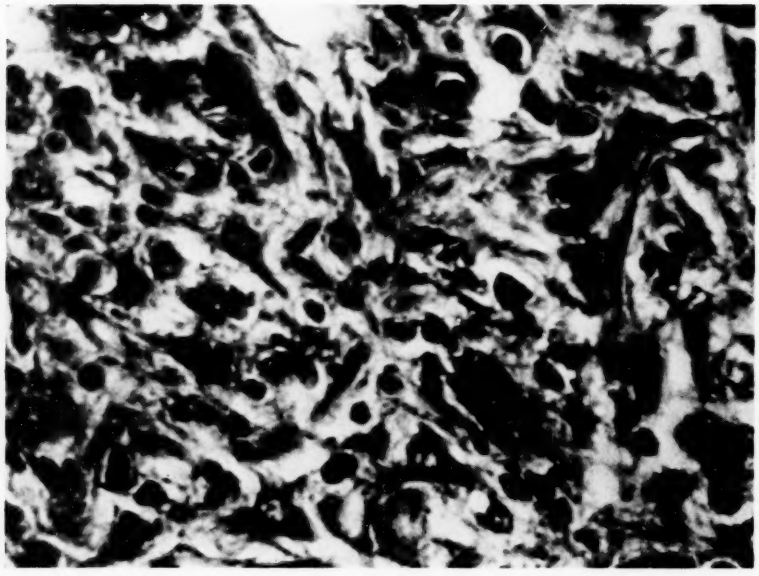

FIG. 2. FIBROBLASTIC AREA IN THE LESION
Some of the cells have a quasi-neoplastic appearance. ×450

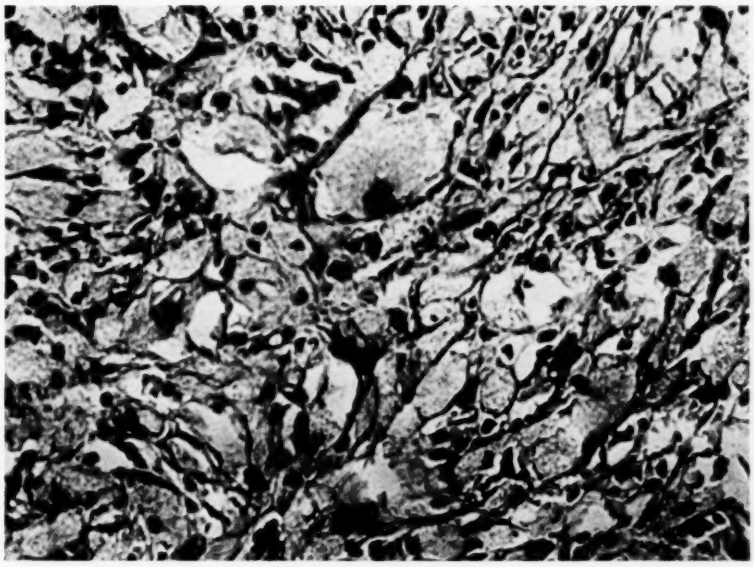

FIG. 3. A FIELD OF FOAM CELLS, OR TOUTON CELLS IN THE LESION FROM A LYMPH NODE. ×220

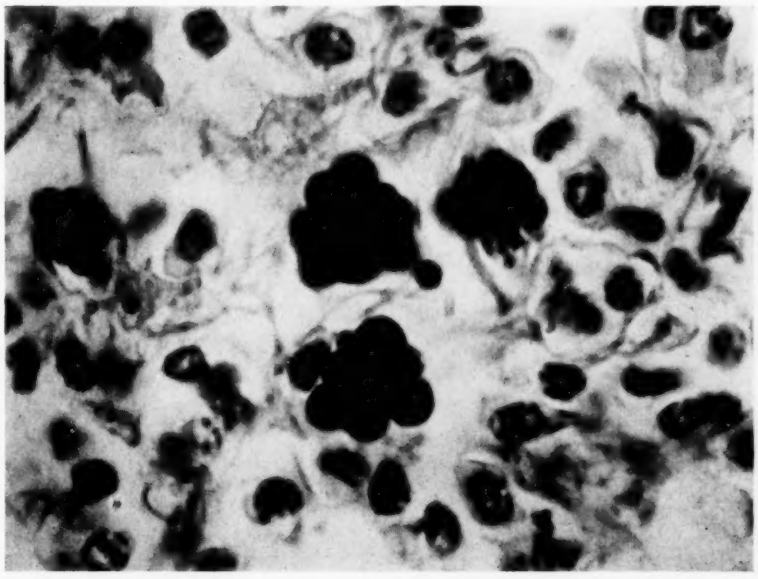

FIG. 4. HIGH POWER VIEW OF SEVERAL SPHERICAL BODIES SURROUNDED BY NUMBERS OF PLASMA CELLS

Gram-Van Gieson. The spheres are intracellular. ×1000

nosed as an unidentified member of the general group of actinomyces by the Central Laboratory. There was no growth on mediums upon which blastomyces and sporotricha flourish, the guinea pig is alive and well at this time.

Pathological examination

Skin lesions: Sections of the biopsied nodes from the skin were found to present pale, fibrous masses of tissue that were confined chiefly to the integument and dipped down slightly into the subcutaneous tissue. They had a yellowish color. No areas of gross pus, nor of caseation were encountered. Microscopically the lesions were seen to be composed of rough, tubercular overgrowths of connective-tissue and retothelium with an unusually large number of giant cells of the Langhans type and many plasma cells. There was some necrosis and numerous miliary foci of polymorphonuclear leukocytes were observed, reminding one somewhat of the lesion of granuloma inguinale, but lacking the definite tubercular grouping of giant cells and the palisaded marginal zones of epithelioid cells seen in that condition. There were often extensive fields of foam, or "Touton" cells. Figures 1 and 2 show the usual appearance of the lesions and figure 3 shows these fields of foam cells, embedded in a rather delicate stroma. These resembled the cells of xanthomas very closely and, with the addition of the giant cells, reminded one of the appearance of xanthosarcoma of tendon sheaths.

Lying free in the tissue, or contained in cells, were perfectly spherical bodies varying from 1 to 15 microns in diameter. When studied in unstained sections with 10 per cent sodium hydroxid they had a yellowish color, were rather refractile and had a suggestion of double contour. They were gram-positive, stained blue with Giemsa's stain, were apparently acid-fast with carbol fuchsin, and stained deep black with iron-hematoxylin. The large forms lay free, the smaller were usually intracellular, constituting masses like clusters of grapes and, strangely enough, contained in plasma cells, which were ordinarily not considered to be phagocytic. There was sometimes a suggestion of buds, but no definite gemma were found. They suggested blastomyces in their general appearance, but lacked the definite double contour and internal structure of these; furthermore, they were never found within giant cells as are blastomyces. Their appearance is well shown in figure 4.

Lymph nodes: The lesions here were in every way identical with those seen in the skin specimens, even to containing the spherical bodies described in them. It made no difference when the lesions were excised, or by whom, they always showed these bodies at all times.

DISCUSSION

The spherical bodies did not resemble inclusion bodies ordinarily seen in virus diseases. They did not grow out in cultures in their spherical form, although somewhat similar bodies did

appear on blood-agar plates taken from another case that presented certain features in common with this one. The patient from whom this material was obtained had had nodules in the skin over a period of years, but these ran a much more acute course and tended to suppurate. The spherical bodies in his biopsies resembled those in the case under discussion in every particular save one, they were acidophil instead of basophil and stained red in Giemsa sections, yellow in Gram-Van Gieson slides and red in Masson trichrome Lichtgrün (instead of black). Basophil bodies of this sort have been seen in small numbers in nasal polyps and other granulomas in this laboratory, but in no other cases excepting one specimen of bone marrow. Very recently they were found in a tonsil that was the site of a rather heavy infestation by ray-fungus of the *Actinomyces hominis* type. The acidophil type was also found in a case of extensive actinomycosis of the buttocks. Naturally these spheres were regarded with frank suspicion and it was believed that they might be merely artifacts from the fixative, water, stains or what not; as they have not materialized in any other tissues examined after being fixed in the same fluids and stained with the same stains, this would seem to rule out such artifacts. Furthermore, they may be observed in the unstained sections embedded in the tissue just as they are in the stained preparations. For this reason and because they were repeatedly observed in all the specimens taken from this patient, it is felt that they must have some significance. It is only in those cases from which actinomyces have been recovered that they occur in any regular profusion. The fact that they are contained in plasma cells, which are not considered to be phagocytes, led me to believe that they might be degeneration products, but the cells in which they lie embedded appear to be in good condition and not degenerating.

The lesion must be differentiated from a number of granulomatous conditions:

(1) It does not appear to be tuberculous. There are no typical tubercles, no typical caseation and no acid-fast bacilli have been demonstrable with carbol fuchsin. Giant cells are far too numerous and scattered, epithelioid cells far too scarce, even for a

diffuse fibrous tuberculosis. The injected guinea pig was alive and well, over two months after the material was injected into it.

(2) It does not appear to be luetic, as the lesion is again too diffuse and the vascular changes are not prominent; the clinical absence of a positive Wassermann reaction is also against this diagnosis.

(3) One might consider Hodgkins' granuloma, but the idea would be soon discarded as there are no Sternberg-Reed cells, no eosinophilia in the tissue and the sclerosis observed would be the only confirmation of such a supposition.

(4) The lesions are quite compatible with some sort of infection with one of the higher bacteria. That it is not blastomycosis of an ordinary type is indicated by the total absence of organism from the giant cells, by the lack of definite double contour to the spheres and by the fact that none grew out on suitable culture medium. Sporotrichosis may be ruled out on a like basis. There were no ray-fungus colonies found in any of the specimens would belie the suspicion that this might be a form of infection by *Actinomyces bovis* or *hominis*.

(5) Pleiomorphic as the nodules of rheumatic fever may be, these lesions do not resemble them; furthermore, the occurrence of such lesions in the lymph nodes would not be in accord with any rheumatic lesion.

(6) There is little in the section to suggest true neoplasm; the lesions are far too heterogeneous in their composition to allow of such a diagnosis, mitotic figures are found in several types of cell, rather than in one type and the fact that the lesions come and go spontaneously is quite unlike neoplasia. The large areas of foam cells suggest xanthoma of some sort, the giant cell xanthoma (or xanthosarcoma) of tendon-sheaths has somewhat this appearance, but xanthomas are often much more similar to granulomas than to true neoplasms, the foam cells being merely phagocytes that have taken up lipins locally.

(7) This leads one to a new group of lesions called "xantho-granuloma" by Oberling, which may be associated with Christian-Schüller disease, or may occur independently. He[1] described some that were strikingly similar to those in this case. He is

inclined to consider our case, which he has seen, as belonging in this category.

One is, therefore, thrown back upon a morphological diagnosis of giant cell granuloma, or xanthogranuloma if one wish to give formal recognition to the foam cells and the yellowish cast of the lesions when observed with the naked eye. How much of a rôle is played by the anaërobic actinomyces in this case we can not say, again we must point to the unscathed guinea pig. The presence of the spherical bodies in the lesions must mean something; it seems highly unlikely that it represents an artifact, still it has been noted in scanty numbers in other granulomas in our laboratory and in one presumably innocent specimen of bone marrow. One must, therefore, maintain an open mind on the subject and wait for more cases of a similar nature before putting too much weight upon the presence of these spheres in the diseased tissue; they may simply represent some form of symbiosis.

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THE QUANTITATIVE ESTIMATION OF BILIRUBIN IN THE BLOOD SERUM OR PLASMA

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The need of a simple, accurate, quantitative method for the determination of bilirubin in the blood is obvious to those engaged in the field of clinical pathology. With the introduction of the icterus index by Meulengracht,[6] it appeared at first that a very simple test was made available. However, experience reveals several technical difficulties inherent in the method. Blood serum especially in hepatic disease is often cloudy because of many foreign substances, particularly lipoids, which make accurate comparison of the serum and potassium dichromate standards impossible.

The van den Bergh reaction which is a specific test for the quantitative determination of biluribin also has inherent technical difficulties. Anomalous factors in the sera reacting with the diazo reagent yield variable shades of yellow or orange color in the alcoholic layer making colorimetric determinations inaccurate or impossible. For this reason, as pointed out by Ottenberg,[7] the test has fallen into disrepute among clinicians.

In view of the admitted fact that the reaction is specific, even though there exist slight discrepancies in the amount of yellow color produced by the direct and indirect reacting bilirubin as pointed out by Elton,[1] experiments were undertaken to solve the difficulty by substituting the Sheard-Sanford photelometer for the colorimeter. A series of cobalt standards were made since the color density falls within the range of the Wratten filter No. 74, and a curve was made as follows:

2.161 grams of anhydrous cobalt sulphate were dissolved in distilled water, diluted to 100 cc. in a volumetric flask and filtered through Whatman filter paper No. 2. To seven centrifuge tubes of 15 cc. capacity, were added respec-

tively, 10, 7.5, 5, 4, 3, 2, and 1 cc. of the standard cobalt solution. To the last six tubes, enough distilled water was added with a pipette to bring the volume up to 10 cc. These tubes were mixed and labelled to read 2, 1.5, 1, 0.8, 0.6, 0.4, and 0.2 mgm. bilirubin respectively. The milligrams when read directly take into account the 7:4 dilution of serum which is used in the van den Bergh method. These seven solutions were read on the galvanometer using the Wratten filter No. 74 in the photelometer with distilled water in the control

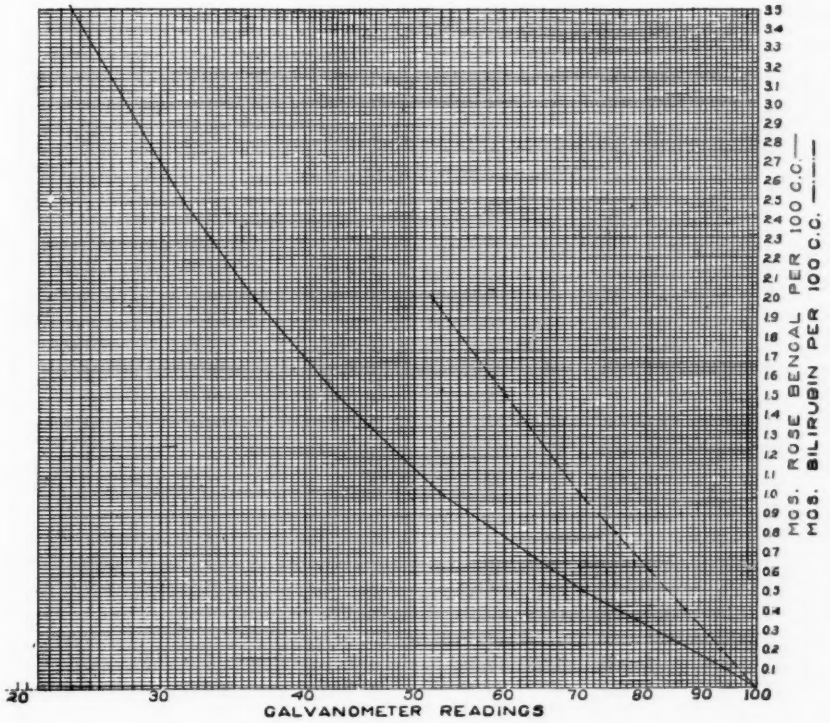

FIG. 1

cup. The points were plotted on the Keuffel and Esser semi-logarithmic paper (1 × 60 cycles) as shown in figure 1. The intermediate points were read off with the aid of a slide rule and a table was made (table 1).

The van den Bergh method with some modifications was used in carrying out the determinations. This method is described by Kolmer and Boerner.[3] In attempting to use this method for

all concentrations of bilirubin, it soon became apparent that plasma or serum of high bilirubin content would yield colors considerably different from the standard and a final dilution of

TABLE 1

CALIBRATION CHART FOR THE QUANTITATIVE DETERMINATION OF BILIRUBIN

PHOTELOMETER READINGS	BILIRUBIN	PHOTELOMETER READINGS	BILIRUBIN	PHOTELOMETER READINGS	BILIRUBIN
	mgm.		*mgm.*		*mgm.*
52.0	2.00	68.0	1.093	84.0	0.490
52.5	1.969	68.5	1.069	84.5	0.477
53.0	1.938	69.0	1.046	85.0	0.458
53.5	1.907	69.5	1.023	85.5	0.439
54.0	1.875	70.0	1.000	86.0	0.418
54.5	1.845	70.5	0.98	86.5	0.406
55.0	1.814	71.0	0.96	87.0	0.387
55.5	1.783	71.5	0.945	87.5	0.374
56.0	1.752	72.0	0.928	88.0	0.362
56.5	1.720	72.5	0.904	88.5	0.342
57.0	1.690	73.0	0.889	89.0	0.329
57.5	1.658	73.5	0.865	89.5	0.310
58.0	1.635	74.0	0.845	90.0	0.297
58.5	1.604	74.5	0.825	90.5	0.284
59.0	1.582	75.0	0.806	91.0	0.265
59.5	1.560	75.5	0.787	91.5	0.252
60.0	1.519	76.0	0.767	92.0	0.232
60.5	1.488	76.5	0.748	92.5	0.219
61.0	1.465	77.0	0.735	93.0	0.206
61.5	1.434	77.5	0.716	93.5	0.187
62.0	1.411	78.0	0.696	94.0	0.174
62.5	1.380	78.5	0.677	94.5	0.161
63.0	1.356	79.0	0.658	95.0	0.142
63.5	1.325	79.5	0.645	95.5	0.129
64.0	1.302	80.0	0.626	96.0	0.116
64.5	1.271	80.5	0.607	96.5	0.096
65.0	1.248	81.0	0.594	97.0	0.083
65.5	1.225	81.5	0.574	97.5	0.071
66.0	1.194	82.0	0.555	98.0	0.059
66.5	1.170	82.5	0.542	98.5	0.046
67.0	1.147	83.0	0.523	99.0	0.0266
67.5	1.124	83.5	0.503	99.5	0.0133

this solution with alcohol would not modify the shade to the extent that the concentration could be correctly determined by the photelometer. After experimenting with variable ratios of

serum to reagent, the following procedure was adopted. The icterus index of the plasma or serum is first roughly estimated, and the following proportions are used accordingly as shown in table 2. In all the above procedures, the serum or plasma is pipetted into a centrifuge tube and the diazo reagent added by overlaying. The reaction is carefully observed, timed and recorded. After one minute the serum and diazo reagent are mixed slightly, the reaction is observed for two minutes, and then finally the serum and the diazo reagent are thoroughly mixed and the tube allowed to stand for ten minutes in which time the reaction is completed.[4] The alcohol and saturated ammonium sulphate are then added in the order given, the solution is mixed well and the tube centrifugalized for ten minutes.

When the bilirubin content of serum is high, the supernatant

TABLE 2

ICTERUS INDEX	SERUM OR PLASMA	DIAZO REAGENT	ALCOHOL 95 PER CENT	SATURATED AMMONIUM SULPHATE
	cc.	*cc.*	*cc.*	*cc.*
5–25	2.0	1.0	6	2
25–50	0.5	0.5	8	2
50–100	0.5	0.5	10	3

fluid usually has a sparkling rose color. When the bilirubin content is below five units the supernatant fluid may show little or no color at all. Ammonium sulphate crystals which occasionally are found on the side of the tube can be removed by centrifugalizing the supernatant fluid. Filtering through filter paper has been found to alter the color of the solution slightly. When serums have a concentration of bilirubin higher than can be read from the chart, the supernatant fluid is diluted with 66.6 per cent alcohol and the mixture centrifugalized to remove unprecipitated proteins which render the solution slightly cloudy. Serums with a low concentration of bilirubin are inclined to be cloudy but the supernatant fluid can be centrifugalized usually with successful results. However, if this procedure does not prove satisfactory, 1 cc. of saturated ammonium sulphate can be added to the supernatant fluid, the solution mixed and the

material centrifugalized. The addition of ammonium sulphate does not alter the color nor the dilution factor. When the solution is clear it is read immediately in the photelometer and the milligrams of bilirubin are calculated keeping in mind the amount of serum used and the dilution factors involved. The azo-bilirubin is dissolved in the alcohol and the saturated ammonium sulphate with the precipitated proteins settles at the bottom of the tube and is not a part of the dilution figure. Serum or plasma may be used as the results obtained by either are essentially the same.

The calculations are based upon the following principle: the final reading in milligrams is equal to the ratio of the dilution of serum in the unknown to the dilution factor in the preparation of the standard, times the galvanometer reading in milligrams. The following is an example of the calculations involved:

If 1 cc. of serum, 0.5 cc. of diazo reagent, 7 cc. of 95 per cent alcohol are used (with 2 cc. of saturated ammonium sulphate to precipitate the proteins) the serum is diluted 1:8.5. The ratio of 1:8.5 to 1:4 (dilution of the standard) is 2.125. Therefore the milligrams of bilirubin in the unknown serum equals 2.125 times the reading in milligrams obtained from the chart. Thus, if the galvanometer reading in the example given is 74 and the bilirubin in milligrams is 0.845, then the final reading is equal to: 2.125 $\times$ 0.845 or 1.79 mgm. of bilirubin per 100 cc. of blood serum.

In conjunction with our quantitative determination of bilirubin using the photelometer, parallel tests were run using the Ernst-Foster[2] method which is essentially as follows:

To 2.5 cc. of serum or plasma, 5 cc. of acetone are added to precipitate the proteins. The mixture is centrifugalized and the supernatant fluid is compared colorimetrically with a 1:6000 dilution of potassium dichromate representing 0.329 mgm. bilirubin per 100 cc. The result is multiplied by 3, the dilution factor.

In our experience, sharper results are obtained when the solution is not filtered as recommended in the original method. When the bilirubin content is low, the serum may be diluted 1:2 with acetone using enough serum to maintain sufficient volume for the colorimeter cups. Serums with an icterus index as low as four units have been estimated quantitatively with little difficulty.

The supernatant fluid is crystal clear after centrifugalizing, but the determinations must be made immediately as the solution becomes cloudy upon standing, or soon after the plunger is lowered into the cup. This difficulty no doubt could be avoided if the readings were obtained by the photelometric method similar

TABLE 3

COMPARATIVE RESULTS

CASE	ICTERUS INDEX ON SERUM	BILIRUBIN BY ERNST-FOSTER	BILIRUBIN BY PHOTELOMETER (VAN DEN BERGH)	VAN DEN BERGH DIRECT
1a	60	5.1	6.33	Positive
1b	100	22.5	21.8	Positive
2	6	1.56	1.17	Negative
3	35	3.9	3.2	Positive
4	120 P	11.8	11.09	Positive
5	4	0.75	0.90	Negative
6	15 P	0.90	1.2	Positive
7	7 P	1.47	1.49	Negative
8	5	0.96	0.95	Negative
9	5	0.84	0.99	Negative
10	30	2.64	2.20	Positive
11a	40	5.6	4.6	Positive
11b	30 F	2.01	2.4	Positive
11c	120	13.2	11.5	Positive
11d	120	14.7	12.0	Positive
12	5 F	0.90	0.90	Negative
13	40 F	3.69	3.4	Positive
14	8 P	1.88	1.74	Positive
15	70 P	14.7	16.4	Positive
16	20	4.44	3.3	Positive
17	7	1.56	1.66	Negative
18	15	2.97	2.86	Positive
19	6	0.82	0.80	Positive
20	4	0.72	0.00	Negative

P indicates poor match; F indicates fair match.

to that described in the van den Bergh adaptation to the Sheard-Sanford photelometer. Such an adaptation of the Ernst-Foster method might even be more simple than the van den Bergh photelometric determination. A comparative study of these parallel procedures is shown in table 3.

It is evident from a study of the figures in table 3 that in

approximately 40 per cent of the cases the icterus index alone did not accurately indicate the bilirubin content of the serum. The comparative determinations by the Ernst-Foster method and the van den Bergh modification run very closely.

For clinical purposes the photelometric determination of bilirubin by the modified van den Bergh method and the Ernst-Foster method are more accurate and easier to determine than the icterus index for a routine method.

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THE INTERPLAY OF HEREDITY AND ENVIRONMENT IN EXPERIMENTAL CANCER*

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In the investigation of the etiology of cancer evidence on the influence of heredity and on the effect of external agents has seemed to come into direct conflict.

Both aspects of the problem have been studied experimentally in the lower animals. The early observations of Loeb suggested that heredity plays a rôle in tumor incidence. This was followed by the work of numerous investigators, Tyzzer, Murray, Slye, Marsh, Strong, Little and his collaborators, Curtis and Dunning, MacDowell, Kreyberg, and others. These authors are not in complete agreement as to the mode of inheritance, the number of factors involved, whether susceptibility is recessive or semidominant or just what is meant by susceptibility, but they do agree on the main thesis that the tendency to develop tumors is inherited.

The experimental proof that extrinsic factors can induce cancer is well known. Fibiger[6] first demonstrated the fact by introducing nematode worms into the stomach of rats and producing carcinoma in that organ. Bullock and Curtis[3,4] induced sarcoma of the liver in rats by infesting them with cestode larvae. Yamagiwa and Itchikawa[23,24] after long-continued painting with tar, succeeded in causing growths in the skin, of the rabbit's ear. Cancer has been elicited by X-ray, and even a normal physiological function such as breeding has been shown experimentally to affect the tumor rate in mice. Female mice which have been bred may have twice as many tumors as their non-bred sisters.[5] Unusually frequent breeding with subsequent pre-

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vention of nursing may raise the rate even higher,[1] while Lathrop and Loeb,[7] and Murray[15,16] have shown that gonadectomy reduces it. Murray went still further and by transplanting ovaries into castrated males caused male mice to develop tumors in the mammary gland. In these cases the effect of the hormones seemed to be upon the growth and development of the mammary gland thus preparing the soil for malignant change. Bogen[2] suggested that the prevention of nursing causes the retention in the breast of secretions which may exert carcinogenic effects similar to those produced by coal tar products.

This line of investigation has demonstrated beyond question the great importance of extrinsic influences in the etiology of tumors. Especially the ease with which tar tumors are produced makes it seem as though any animal would succumb to the irritation and that heredity is unimportant. It challenges the results of the geneticists. Data accumulated in our laboratory have a direct bearing on this point and in fact, reconcile the apparently conflicting points of view.

For some time we have had under observation a colony of mice which produce spontaneous tumors. The animals are kept under as nearly normal conditions as is possible in a laboratory. They are fed a standard diet, their histories are recorded, their relationships known, and at death necropsy is performed on every individual. Sections are taken of suspicious nodules.

In a general survey of the colony it was found that a number of tumor types were represented. The predominant one, however, was found to arise in the lungs, and it is chiefly growths in this organ that supply the evidence to be reported here.

Histologically these lung tumors are similar to those which have been described by Livingood,[9] Tyzzer,[20] Slye, Holmes and Wells,[18] Schabad and others.[17]

Figure 1 illustrates a common type. It consists essentially of epithelial cells situated upon thin folds of supporting tissue. However, there is a great variation in the microscopical picture. Sometimes the spaces between the plications are so great as plainly to suggest a cystic structure. In other instances the cells are more compactly arranged in a connective tissue stroma.

The cells may be columnar, cuboidal, or resemble thickened alveolar epithelium; they vary in size and mitoses are infrequent. The tumors increase by internal growth or by extension into the alveolar spaces. They sometimes invade the bronchi, more rarely, the lymphatic and blood vessels. Secondary growths frequently appear in the lung, occasionally in the chest wall,

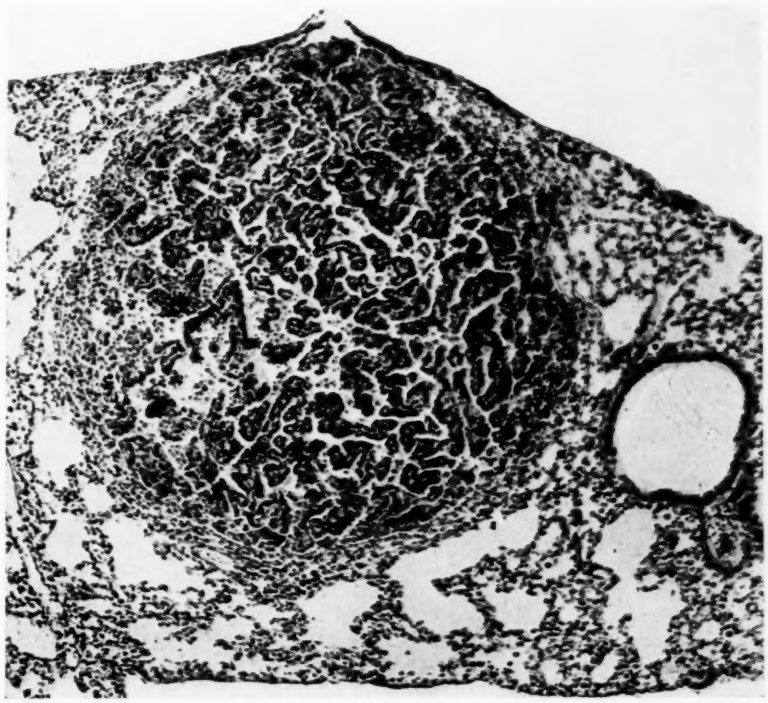

FIG. 1. SPONTANEOUS TUMOR OF THE LUNG IN A MOUSE. ×96

mediastinum, and diaphragm and rarely in more distant organs such as the liver or kidney. These tumors range from benign growths, which are often difficult to distinguish from simple hyperplasia, to truly malignant neoplasms. Several types may occur in the same animal. For this study no attempt has been made to classify the several types. They are all treated on the same basis.

INFLUENCE OF HEREDITY

The mouse colony is composed of a number of strains from various sources and it was noted that the tumors were not uniformly distributed among them.[10,11,12] Some strains had higher rates than others and the difference between them was often greater than could be accounted for by chance. As an illus-

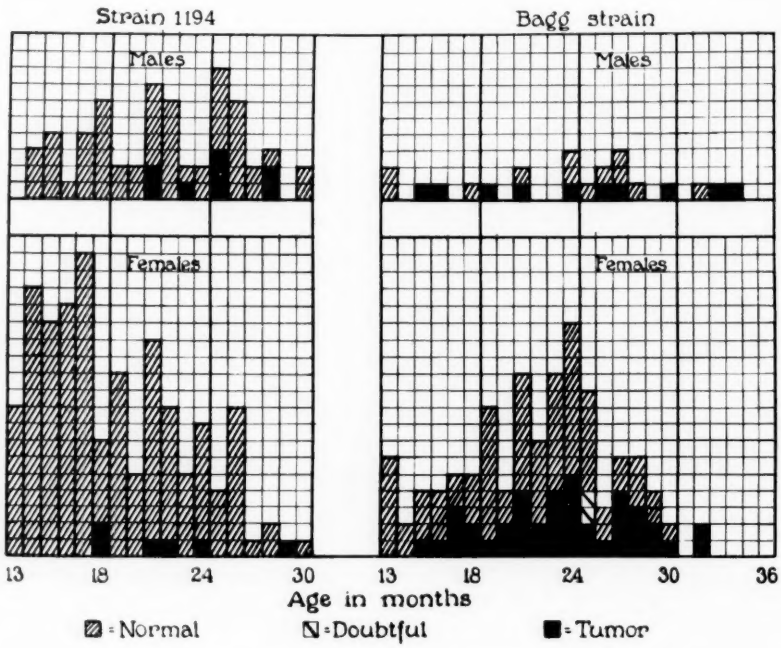

FIG. 2. COMPARISON OF STRAIN 1194 WITH THE BAGG STRAIN

Each mouse is represented by a block located on the chart according to age in months at time of death.

tration a comparison may be made between strain 1194 and a branch of the Bagg albinos (fig. 2). The former mice are colored, mostly black agouti, like the wild house mouse. The 208 individuals composing the group were all descended from one pair and include about ten generations, usually produced by brother-sister matings. The 137 albinos belonged to a strain which has been inbred for a number of years but not always brother by sister.

Since age affects the tumor rate, an analysis of the data has been made for six month periods. Table 1 shows striking differences between strains at all age periods. The total incidence of lung tumors for strain 1194 in mice over 12 months is 6.7 per cent and for the albinos, excluding the two doubtful cases, 37.0 per cent. A mathematical calculation shows that, considering the number of mice concerned, the probability of a difference as great as this occurring by chance is negligible.

These rates are maintained when the animals are housed together (table 2). There are records of fifteen cages containing mice derived from these two strains. Three to five animals from

TABLE 1

COMPARISON OF LUNG TUMOR INCIDENCE IN TWO STRAINS OF MICE

AGE	STRAIN 1194			BAGG STRAIN				DIFFERENCE	DIFFERENCE / P.E.
	Without lung tumor	With lung tumor	Per cent tumor	Without lung tumor	With lung tumor	Doubtful	Per cent tumor		
months									
13–18	95	2	2.1 ± 1.0	22	9	0	29.0 ± 5.5	26.9 ± 5.6	4.8
19–24	66	6	8.3 ± 2.2	41	21	0	33.9 ± 4.0	25.6 ± 4.6	5.5
25–36	33	6	15.4 ± 3.9	22	20	2	47.6 ± 5.2	32.2 ± 6.5	5.0
Totals.....	194	14	6.7 ± 1.2	85	50	2	37.0 ± 2.8	30.3 ± 3.0	10.1

each family had been placed in the same box as they became available at four weeks of age or a little older and they remained together the rest of their lives. The length of this period was not uniform, but more than half of the colored mice lived in association with the Bagg strain for a year to a year and a half. Only males were used so that no allowance need be made for any possible variation attributable to sex. No tumor was found in any organ except the lung. Since eleven months was the earliest age at which any individual was found at necropsy to have a tumor, the incidence is calculated from that age. Among forty-three colored mice that lived eleven months or more only two had lung tumors (4.7 per cent) while among fifty Bagg mice of

TABLE 2

List of Cages Containing Mice from Two Different Strains

Cage number	Mice indicated by age in months at time of death		Cage number	Mice indicated by age in months at time of death	
	Strain 1194	Bagg albino		Strain 1194	Bagg albino
4464	6	10	4541	12	13
	11	19		13	13
	16	21		13	17 P
	18				19
	23				19 P
4465	8	9	4577	9	9
	9	9		10	12
	10	10		11	12
	24	12			13
					14
4467	17	11 P			
	17	18	4579	8	12
	18	19		8	13
	19	19		8	13 P
				17	14 P
4472	4	17 P		18	
	18	19			
	22	19	4580	9	7
	22	22 P		12	7
				12	7
4494	14	14		20	16
	15	15			20
	15	16 P			
	17 P	19 P	4590	2	12
	22 P			16	14
				16	15 P
4538	6	9			16 P
	12	9			17
	15	15			
	16	15	4591	11	8
		18 P		16	18
				17	18
4539	5	8		18	19
	7	13		20	19 P
	12	13 P			
	14	14	4593	11	6*
				14*	6*
4540	5	14 P		15	6
	5	14		15	15 P
	6	15		16	
	6	15 P			
	14				

P = pulmonary tumor.

* No necropsy.

corresponding age seventeen were tumor-bearers (34.0 per cent). The rates are characteristic of the strains from which the mice came. In this case, as far as can be determined, the environ-

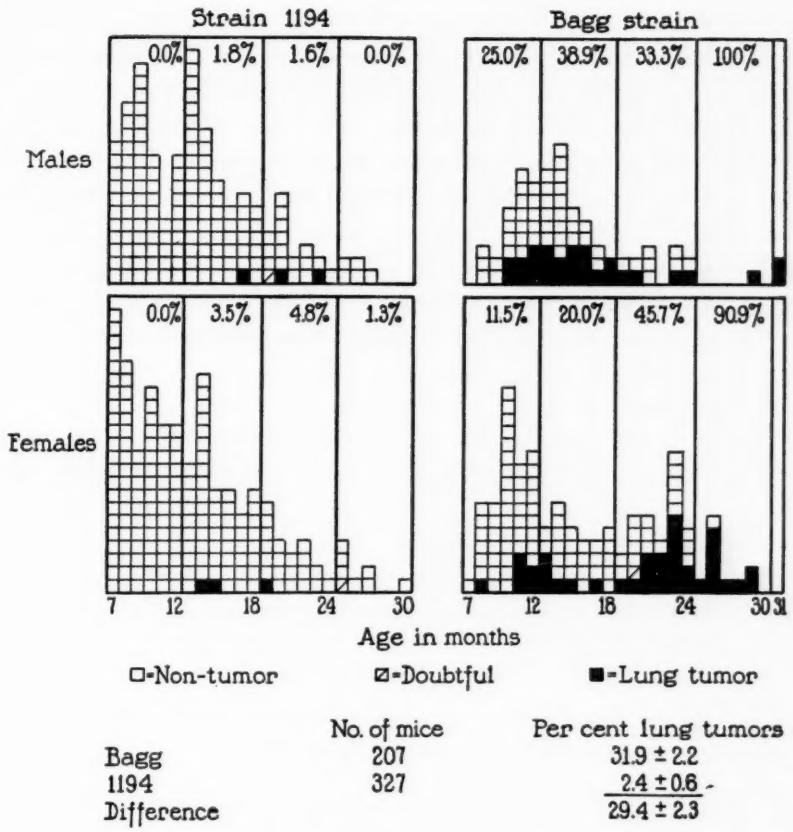

FIG. 3. LUNG TUMOR PERCENTAGES IN RECENT GENERATIONS (MAY 1931–DECEMBER 1934)

In the Bagg strain doubtful cases were regarded as negative and in strain 1194 as positive.

mental conditions were identical, apparently the only difference between the groups was their inheritance.

It is some time since these data were collected and it is of interest to know whether the differences persist. The strains

have been continued in a number of lines and figure 3 records the performance of these same stocks during the last four years covering from six to eleven generations. In the Bagg stock the tumors appeared earlier so that the data are given for mice over six months of age. The same difference may be observed between the strains. The totals show that the Baggs had 31.9 per cent of lung tumors and the 1194 had 2.4 per cent, a difference of 29.4 per cent, about the same as before. If the calculations include only mice over a year old an incidence of 41.7 per cent is found for the Baggs and 4.8 per cent for strain 1194, the differ-

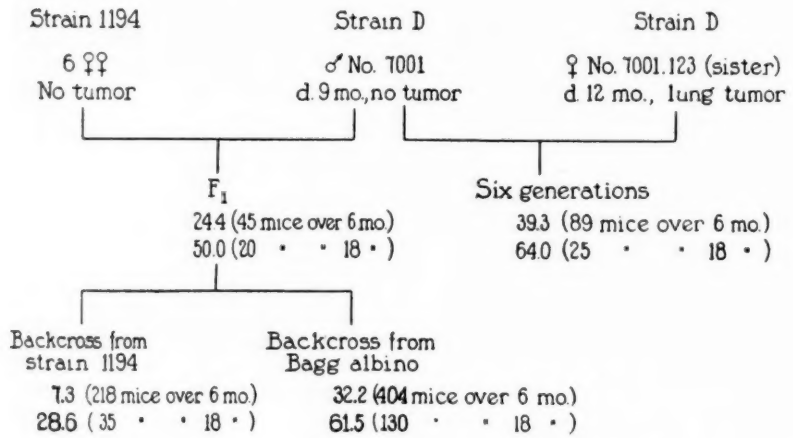

FIG. 4. SHOWING THE PERCENTAGE OF SPONTANEOUS LUNG TUMORS AMONG DESCENDANTS OF MALE 7001

ence is 36.9 per cent. These tumor rates are therefore not of sporadic occurrence, but have been consistently maintained over a number of years.

This difference between strains is ascribed to heredity because the members comprising the respective groups are related through descent. But these data might be compared to circumstantial evidence. The Mendelian test of heredity is a breeding test, where individuals of opposite type are crossed. The hybrids thus produced are either inbred or crossed back with the original strains. In the second cross, if the character is hereditary, the

genes which mingled in the hybrid segregate out, giving different percentages of the character in different classes. Figure 4 illustrates such a test cross.

The male no. 7001 came from strain D. This is another albino stock with the same rate (37 per cent) as the high tumor strain just described, but the tumors appear earlier. Although the male died without having a tumor, nine months is under the average tumor age in this stock. Mated with a sister that had a lung tumor at twelve months, it produced fourteen young, of which two, possibly three had lung tumors, that is to say, lung tumors appeared in the first generation. These descendants were inbred in various ways and among eighty-nine individuals that lived more than six months, 39.3 per cent had lung tumors. Male 7001 was also crossed with six females from the low tumor strain 1194. Although the females themselves were tumor-free and in addition, came from a low tumor line, all except one had some offspring with tumors. The exceptional one had only two daughters that lived into tumor age, too small a number to be a genetic test. The total rate for the hybrids was 24.4 per cent for mice over six months old. The appearance of tumors in the first generation of the cross suggests that susceptibility is dominant, or rather semidominant since age and other influences must prevent the somatic expression of the tumor character in many individuals which are genetically "tumor mice." That the genetically "non-tumor mouse" cannot be identified by simple inspection constitutes one of the major difficulties presented by this problem. Tumors may arise at practically any age. They may be seen in mice as young as two to three months or at any time thereafter, but there is no critical period at which the growth must appear if the individual has the genetic potentiality of producing one. Though a mouse dies at three years of age without a tumor it is not certain that if it had lived a month longer a tumor might not have arisen.

In continuing the experiment mice of the hybrid generation were crossed back to the two parental stocks. Forty-two additional females from strain D were used as backcross mothers. Among those living more than six months 39 per cent had tumors.

TABLE 3

OFFSPRING FROM MATINGS BETWEEN TWO TUMOR AND TWO NON-TUMOR PARENTS

♂ NO TUMOR 26 MONTHS × ♀ NO TUMOR 36 MONTHS		♂ LUNG TUMOR 34 MONTHS × ♀ LUNG TUMOR 22 MONTHS				♂ LUNG TUMOR 30 MONTHS × ♀ LUNG TUMOR 20 MONTHS	
		Sons		Daughters		Daughters	
Without lung tumor	With lung tumor	Without lung tumor	With lung tumor	Without lung tumor	With lung tumor	Without lung tumor	With lung tumor
11		7		7		16	
13				8			20
13				8			21
17		11				22	
17				12			27
19				16			27
23				16			29
23				16 M		37	
24	24			17 M			
	24				18		
	24	19	19	19			
	25 ♂			20			
	25	21		21	21		
26	26			22			
	26			22 M	22 M		
	26			23			
	27	24		24			
	27			24			
	27			24			
	27			25			
	28 M		26	26	26		
	28			26			
29	29			26 M			
	29	27					
30		27					
31	31				28		
31	31				30		
31				31			
	32	32			32		
33							
33	33						
	34						
	34						

Mice indicated by age in months at time of death. M = mammary tumor.

Among thirty-nine backcross mothers from the low tumor strain none had tumors. In the resulting generations the two groups of offspring had very different tumor rates. The backcross to the high strain produced 404 mice that lived more than six months. Their tumor rate was 32.2 per cent. The backcross in the other direction gave 218 mice with a rate of 7.3 per cent. These data, checked for age and sex, were found to represent real differences between the classes, and are interpreted as being due to the segregation of Mendelian factors influencing tumor susceptibility.

The question of dominance forms part of the genetic problem of the mode of inheritance. According to the Mendelian theory, the simple recessive individual mated with the recessive should produce only recessives. However, in this work, since the tumor

TABLE 4

PERCENTAGES OF LUNG TUMOR FROM DIFFERENT TYPES OF MATING

TYPE OF MATING	NO TUMOR	TUMOR	PER CENT TUMOR
O X O	379	86	18
T X O	124	81	40
T X T	32	29	48

O X O = neither parent with tumor.
T X O = one parent with tumor.
T X T = both parents with tumor.

character, as just stated, is variable in its expression, this test for the recessive type fails. An examination of progeny from similar parents (table 3) shows that whether the two parents are positive or negative, both tumor and non-tumor offspring may be produced. In one experiment one daughter from tumorous parents lived to thirty-seven months without being affected. This emphasizes again the variability of the character under observation.

Although a comparison of the offspring from matings of different types does not reveal the recessive, if a sufficient number of examples is taken it is found that the kind of parentage affects the total proportion of tumor-bearers among the descendants. In data from mice of mixed ancestry (table 4) crosses between

non-tumor parents gave 18 per cent of lung tumors, when one parent was affected the tumor rate was 40 per cent and when both were tumorous it was 48 per cent The progeny from tumor-free parents had a significantly lower incidence of lung tumors than those from matings where one or more parents had tumors.

In the discussion so far three kinds of evidence have been presented: (1) it has been shown that inbred strains of mice have different tumor rates; (2) that a cross between such strains gives evidence of Mendelian segregation; (3) that different kinds of parentage affect the tumor rate of the offspring. On the basis of these findings the influence of heredity on lung tumors in mice seems fully established.

INFLUENCE OF THE ENVIRONMENT

That external factors can cause tumors in mice was demonstrated by Tsutsui.[19] By painting the skin with tar he produced epithelioma. Later Murphy and Sturm[14] discovered that tumors were produced in the lung by cutaneous tarring. The irritant used in such experiments is made from the residue from coal tar which has been heated to 377°C. The final preparation is a benzene extract from which the acids, bases, and phenols have been removed. The tarring differs but slightly. To induce skin tumors, the irritant is applied always to the same area in the interscapular region, three times per week for four months. Sometimes before the end of tarring, but usually after a latent period, tumors may appear in the skin, and at necropsy, under certain conditions, nodules may be found in the lung also. To induce lung tumors only, the same number of applications is given over the same length of time, but they are distributed to twelve different areas consecutively. No spot is tarred more than four times. Usually that is not sufficient to elicit tumors in the skin. The mice are allowed to live six months longer and then all killed at the same time so as to eliminate variations due to age. They are then twelve to thirteen months old. This procedure induces tumors in the lungs, although the exact mechanism by which this is accomplished is not clear.

Both methods were used to investigate the type of response

given by inbred stocks of mice. The lung tumor technique was applied to two of the previously mentioned strains known to differ in the number of lung tumors which they produce under ordinary laboratory conditions. After tarring, the tumor incidence of both strains went up, but not to the same level (table 5). The rate of the colored strain increased from 6.7 per cent to 22.4, but the Bagg albinos which before had shown 37.0 per cent gave 85.4 per cent. The difference between them had been increased, being twice as great as it was before. The stock which was less susceptible to spontaneous growths in the lung was also less susceptible to tar-induced lung tumors.

This difference in response to tarring is a constant charac-

TABLE 5

SHOWING PERCENTAGES OF LUNG TUMOR IN TWO STRAINS OF MICE WITH AND WITHOUT CUTANEOUS TARRING

	STRAIN 1194		BAGG STRAIN	
	Number of mice	Per cent lung tumor	Number of mice	Per cent lung tumor
Original stock (mice 12–34 mos. old).....	208	6.7	135	37.0
First tarred group (mice 12–13 mos. old)...	49	22.4	48	85.4
Subsequent tarred groups (mice 12–13 mos. old).....	199	14.6	395	92.9

teristic of these stocks. For the last six years, descendants of some of these individuals have been tarred, in some instances for eleven generations. An attempt has been made to select toward more and less susceptible lines. One hundred and ninety-nine mice of the colored strain have given 14.6 per cent lung tumors, while 395 albinos have given 92.9 per cent.

As in the case with spontaneous tumors a cross was made this time to see if susceptibility to tar-induced tumors is inherited. Individuals from the groups that later gave 22 and 85 per cent tumors after tarring were mated and the hybrids produced by them were also tarred (fig. 5). Again in the first filial generation the incidence was high (78.6 per cent) suggesting, though not

proving, that susceptibility is semidominant. When the hybrid generation was crossed back to the high strains the rate remained high (81.1 per cent). When the backcross was made to the low strain it dropped half (39.5 per cent). The difference between them is six times its probable error and is mathematically significant (41.6 ± 6.9 per cent). Evidently the tumor rate depended on the family to which the cross was made. These results parallel those obtained with spontaneous tumors showing that susceptibility to induced tumors also is subject to genetic control.

Because of the possession of methods of inducing tar tumors in either the lungs or skin an opportunity was afforded of investi-

	STRAIN 1194	BAGG ALBINO	DIFFERENCE	DIFFERENCE P.E.
Per cent spontaneous lung tumor in mice over 12 months	6.7 ± 1.2	37.0 ± 2.8	30.3 ± 3.0	10.1
Per cent lung tumor after cutaneous tarring in mice 12 to 13 months	22.4 ± 4.0	85.4 ± 3.4	63.0 ± 5.3	11.9
	F_1 78.6 ± 5.2			
	Backcross from Strain 1194	Backcross from Bagg albino		
	39.5 ± 5.3	81.1 ± 4.3	41.6 ± 6.9	6.0

FIG. 5. SHOWING PERCENTAGE OF TAR-INDUCED LUNG TUMORS IN STRAIN 1194 AND THE BAGG STRAIN, AND IN THE HYBRID AND BACKCROSS GENERATIONS

gating the problem of specificity, of discovering whether the inherited tendency to develop tumors is a general one or whether a separate mechanism governs particular organs.

In order to throw light on this point, strains were tested for their susceptibility to skin tumors. Five groups of mice were selected. There were eighty Bagg albinos and 100 mice from the dark colored strain used in the previous experiment. There was another lot of twenty-three Bagg albinos descended from a different inbred line. Seventy-one mice were taken from strain 62, also highly inbred and in color, pink-eyed, dilute brown. The last group of fifty-four animals were of somewhat mixed ancestry, but all were alike in possessing the dominant hereditary character of

hairlessness, known technically as "naked." Hair is formed but imperfectly so, and breaks off, leaving only an occasional patch of fur. To the mice in the five groups tar was applied, always on the same spot in the interscapular region, three times per week for four months. The course of the experiment may be followed in figure 6. There were marked differences in response. Strain 62 was the most susceptible. After 250 days about one-half of the individuals had developed epithelioma, but the mortality from intercurrent disease was very high and no mouse survived

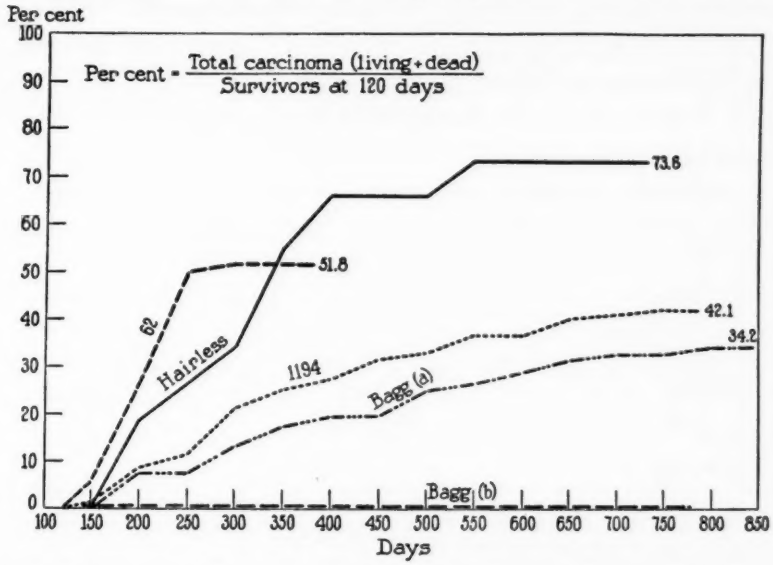

FIG. 6. SHOWING THE PERCENTAGE OF CARCINOMA OF THE SKIN IN FIVE GROUPS OF MICE SUBJECTED TO CUTANEOUS TARRING

400 days. The hairless were almost as susceptible as shown by the early part of the experiment. They lived longer and the final figure was actually higher (73.6 per cent). The dark mice and the albinos reacted about the same, giving 34.2 and 42.1 per cent respectively, although, it will be remembered, 1194 is the strain that has few lung tumors, either spontaneous or after tarring, while the Baggs have a high percentage of lung tumors. The small group of twenty-three Bagg albino mice had no tumors at all, although they lived to be quite old.

A comparison of the susceptibilities of these strains with respect to the two kinds of tumors is given in table 6. The lung tumor rates shown are for mice living 300 days or more. Strain 62 is omitted because the majority of these animals died before that time. It is interesting to note that the hairless mice are highly susceptible to tumors of both types. While strain 1194 had a moderately high skin tumor incidence but a low lung tumor rate, the Bagg (a) had a moderately high skin tumor incidence but a high lung tumor rate. The smaller group (b) of Bagg mice was highly insusceptible to skin tumors but showed a high percentage of tumors in the lung. There is evidently no correlation between the tumor rates. Resulting from an identical stimulus, tumor formation in the 2 organs is determined separately. It

TABLE 6

COMPARISON OF TUMOR SUSCEPTIBILITIES (SKIN TUMOR TECHNIC)

Total percentage of skin tumors among all mice surviving treatment (120 days). Percentage of lung tumors among mice living 300 days or more.

STRAIN	SKIN TUMOR	LUNG TUMOR
Hairless	73.6 ± 4.1	73.8 ± 4.6
1194	42.1 ± 3.4	16.2 ± 2.9
Bagg (a)	34.2 ± 3.7	81.5 ± 3.2
Bagg (b)	0.0	80.0 ± 6.0

is to be noted that in using external agents in inducing skin tumor certain variables are encountered, for example, the removal of the tar by the mouse itself. Therefore, an attempt is being made to evaluate the bearing of mechanical, chemical or physiological factors, on the observed strain differences.

It seems possible to draw certain conclusions from the experiments which have been described: (1) external agents cause cancer in mice; (2) such agents have their limitation, and the limiting factor is the constitutional type of the individual. Certain incitants applied to a susceptible animal result in tumor growth, but the same amount of stimulation applied to an insusceptible animal may fail entirely in its effect; (3) these constitutional differences in susceptibility are inherited and (4) susceptibility is apparently organ-specific.

APPLICATION TO HUMAN CANCER

Since biological principles discovered in lower mammals are applicable to higher organisms, it is pertinent to inquire whether there is any confirmation of these experimental findings in human cancer. There is no need to rehearse the evidence for the influence of external irritants. At least in certain types of neoplastic disease their action is striking. Descriptions of kangri cancer, chimney sweeps' cancer, and occupational cancer are familiar to the medical profession. It may be emphasized, however, that not all chimney sweeps get cancer nor do all pipe smokers develop carcinoma of the lip or tongue even after what seems to be sufficient provocation. One may suspect differences in individual resistance due to descent.

In regard to heredity the evidence is varied. Little,[8] Waaler,[22] and others have concluded upon the basis of statistics from large populations that heredity plays an important part. However, doubt as to the reliability of the data composing mortality statistics has weakened the confidence in their use for comparative purposes. In all probability in the statistical approach to the problem the situation has been confused partly because of the difficulty of separating the effects of the environment from those due to heredity. A person who does not develop cancer may owe his immunity either to freedom from contact with cancer-inducing agents or to an hereditary insusceptibility.

A further point to be noted is that the two factors of heredity and environment may not be active in the same ratio in all types of tumor. In some classes heredity may have more weight than in others.

Perhaps the most clear-cut case showing the influence of heredity is that of glioma retinae. The fact that it occurs in infancy helps in eliminating the variability due to the time factor. It has a very high familial frequency. The list reported by Macklin[13] shows the high percentage of patients in affected families. Out of a total of 144 children listed in her table, eighty-one suffered from the tumor. The list includes not only the well-known case of Newton in which ten out of sixteen children died of retinal glioma and of Wilson in which a family of eight

was afflicted, but also a large number of instances showing the concentration of the disease in families. It is obvious that parents with one affected child run great risk in having more offspring.

As an illustration in which the operation of both heredity and environment can be seen we may cite xeroderma pigmentosum. The character which is inherited is a hypersensitivity of the skin which under the influence of light undergoes malignant change. Since the incitant (sunlight) is universal, the rôle of heredity can be easily traced. Most cases seem to be due to a simple recessive factor.

In von Recklinghausen's disease and cartilaginous exostoses the environmental influences apparently are sufficiently constant so

TABLE 7

SHOWING THE OCCURRENCE OF TUMORS IN MONOZYGOTIC AND DIZYGOTIC TWINS

	BOTH TWINS HAD TUMOR		ONE TWIN HAD TUMOR
	Same organ affected; same type tumor; at about the same time	Different organs affected	
Monozygotic	21 (26) 51 per cent	0	20
Dizygotic	3 10 per cent	8	20

that the hereditary tendency can be detected although there is reason to believe that environment is effective to some extent. The inheritance of these diseases apparently conforms to a simple type.

At the present time a good deal of interest is manifested in data from identical twins. In the literature may be found reports of at least forty-one cases. In five additional accounts the evidence for monozygocity was doubtful. Also examples of naevae and haemangioma which have appeared in certain lists have been excluded. If heredity were the only factor involved since both members of the pair are from the same fertilized egg, both should show the same hereditary disease. In classifying these cases, (table 7) in twenty-one (possibly twenty-six) both individuals of

a pair had tumors of the same type, in the same organ, and at approximately the same time. Although in two instances one partner had an additional growth in a second organ, no cases were found in which two afflicted partners had a totally different history. There is another class of twenty cases in which only one member of the pair had a tumor, but since in many instances the other member was still living at the time of observation, the data are incomplete. An examination of the list of twins from two fertilized eggs reveals that only three instances have been reported where the cancer history was similar. In eight cases both members were affected, but the tumors were not in the same organ; in twenty cases only one member was affected. Concordance is shown in only 10 per cent of the dizygotic twins, but in 51 per cent of monozygotic twins. However, sweeping conclusions should not be dawn from this limited sample. It may be questioned whether the data are truly representative since they include records of isolated cases. Striking cases are more apt to be reported while contrary evidence is disregarded. Nevertheless it would seem to be highly significant that no case of identical twins has been recorded in which the tumors appearing in both were totally different. The method of using evidence from twins should be a fruitful one and a large amount of data impartially collected from a given population should be of great value.

It seems likely that each type of tumor should be treated separately. In the data under consideration the disease occurred in a wide variety of organs: bone, breast, brain, eye, lip, kidney, ovary, rectum, stomach, testis, uterus. The class of unioval twins with only one member affected included one case of von Recklinghausen's disease. Although this affliction is regarded as hereditary, it is also influenced to some extent by environment and here is one instance in which the partner survived twenty years. In regard to carcinoma of the stomach, one case occurred in which both partners were affected, but in three pairs only one partner showed it. One unaffected partner survived twenty-one years. Here apparently we are dealing with a type of tumor in which, although differences in susceptibility due to heredity may

be expected, environmental influences play a considerable part in its manifestation.

For the majority of tumors little is known concerning the relative importance of extrinsic and intrinsic factors. There is a pressing necessity for the extension of cancer research in the field of prevention. Identification of the substances, conditions, habits which lead to carcinoma and the consequent possibility of their avoidance, might well contribute towards checking the rising cancer death rate. In a system with two variables, information about one of them will be more readily obtained if the other is kept constant. Genetically controlled material is a prerequisite for experiments in this type of work. The results will be vitally affected by the hereditary constitution of the host. It is necessary to know whether or not the animal being used as test material is susceptible to the kind of tumor under investigation. A negative result might be due to the resistance of the animal rather than the harmless nature of the treatment.

Investigations of this sort not only promise to yield practical information but a knowledge of the operation of extrinsic and intrinsic factors will contribute to our fundamental understanding of the nature of cancer.

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EDITORIAL

Hormones and Carcinogenesis

The part played by hormones in cancer is becoming of great practical interest, and it seems necessary to try to catalogue the literature as it arrives into some sort of order. It is all the more necessary because reports are often contradictory, and many of them do not seem to carry their logic back far enough to fundamentals of biology.

Among others, we may ask two questions: (1) do cancers produce hormones and (2) do hormones produce cancer? These two questions are often confused, but are in reality quite distinct in their experimental results, their observational facts and their implications. The latter question is by far the more difficult of evaluation.

Reverting to fundamentals, the ability of cancer cells to differentiate is disturbed and they have no organizing powers at all in the ordinary sense of the word. But to produce hormones, cancer cells must differentiate, at least to the degree which is called "chemical differentiation." All cells have certain chemical activities in common such as respiration; as they differentiate they develop more specialized reactions in addition. These, called "chemical differentiations," are illustrated by the ability to produce saliva by salivary cells, thyroxin by thyroid cells, et cetera. That the cells of certain cancers reach a point in their differentiation where they produce hormones has been shown abundantly in a few tumors such as the embryonal adenocarcinoma of the testis wherein a hormone has been identified and has even been used for diagnostic purposes as it appears in the urine. When the tumor is removed the excretion of hormone ceases. When metastases develop, the secretion reappears.

The answer to the first question, then, is "yes" from direct evidence. Certain cancers do produce hormones. Incidentally

if one could find some specific secretion of other cancers perhaps one could diagnose them too by finding the results of the "chemical differentiation" of their cells in the blood, urine or some other fluid.

The second question, do hormones produce cancer, requires consideration of two contrasting possibilities as assayed in experimental embryology.

The first is: cells, that is, their protoplasm, contain within themselves the potentialities of differentiating into a number of different kinds of units, and the environment, in the broad sense of the word, determines which of the possibilities is to be realized and also to what degree. The second is: cells have no specific, only vague general potentialities and are molded into their specific differentiations entirely by their environment.

Experimental facts can be marshalled to favor both views. In any case, species specificity is not impressed from without, and at least this restriction to unlimited possibility is contained within cells.

Regarded from the evolutionary point of view, as far as growth and development are concerned, hormones have been evolved to guide differential growth so that the various organs and parts of a complete organism maintain their correct masses relative to each other. Even if the potency of protoplasm is limitless, and its differentiations are molded entirely by the environment, its expressions, nevertheless, must be limited, if it is to be gathered within an effective organism. And if, on the other hand, the potencies of protoplasm are self-contained and the environment only determines which of a number of choices shall be made, they still must be limited by the whole organism. In either case if hormones regulate differential growth, they do it by playing upon cell potencies, so to speak, now allowing them to expand, now keeping them contracted. Mention need be made of only one example among many, that of the way expansion and contraction of cell growth and development take place in the breast under the influence of ovarian hormone. It is critical for this argument that these proliferating and differentiating cells produce breast tissue and not something else. But what of experi-

ments such as Lacassagne's in which certain male mice did develop cancer of the breast after continued injections of ovarian hormone? They came from a "cancer strain!" Thus, in addition to the hormone injections, there was this "constitutional" predisposition in the picture.

Other things being equal, it is a fair assumption that the more cells that are actively dividing, the more statistical chance there is for one or several going wrong in their subsequent differentiation. Hormones produce this cellular proliferation and thus give more opportunity for the altering of potencies to take place which results in cancer, but so also does the short, hot clay pipe stem which produces cellular activity on the lip which in turn, later, leads to cancer.

For the present it seems, until more work is forthcoming, that "cancers produce hormones but hormones do not directly produce cancer."

—Stanley P. Reimann.

NEWS AND NOTICES

The rapid development of photographic processes for making records has made it necessary that organizations publishing journals should carefully consider the present trends. A number of organizations are offering to scientists photographic copies of articles which appear in journals and books. Perhaps the first of these beginning this service was the Library of the Department of Agriculture in Washington and they have formed an agreement with Science Service which, under the name of Bibliofilm Service, has indicated that it is in a position to supply scientists with either 35 millimeter copy or photo-prints at a nominal sum. The effect on publications and the sale of back copies of journals is at the present time very problematical. The Bibliofilm Service has indicated that it will not photostat recently copyrighted material. However, no further elucidation of this statement has been made by them. There is certainly a difference of opinion as to the legality of the whole procedure and this will probably not be settled until some test case is brought to court. It is easy to see, however, that if scientists can procure for a few cents copies of articles published in journals, it will eventually hurt the sale of the journals. It will undoubtedly destroy much of the value in back numbers. The attention of the Society is called to this new development and it is hoped that members will give serious consideration to the possible effects on this JOURNAL.

There is another feature of the Science Service proposition which demands attention. It is urging editors to send them manuscripts which cannot be published and are willing to accept manuscripts from authors. These will be copied on 35 mm. film and will be available to scientists who desire to purchase them. It is expected that these articles will be listed and abstracts may even be arranged for. The effect of this on the availability of material for journals is again not clear but the effect on priority is certainly a serious one. It will have to be decided whether such

reproductions constitute publication. Some experts in zoology and botany have already expressed themselves to the effect that so far as descriptions of new species are concerned, this will not constitute legal publication. On the other hand, there will be little inducement for an editor to publish articles which have already been photographed and listed and this again is a serious problem for editors to decide.

This whole problem is not one peculiar to the American Society of Clinical Pathologists but involves the entire field of scientific publication.

A preliminary announcement of the Harvard Tercentenary Conference of Arts and Sciences has been made. It will be held from August 31 to September 12, 1936, in Cambridge. The preliminary program indicates that this will be the most significant conference on Arts and Sciences held during this generation, indeed, if not the most significant held during the present century. A perusal of the subjects and speakers indicates the wide scope of the conference and the fact that the leading men in their fields throughout the whole world will be present.

BOOK REVIEWS

Post Mortems and Morbid Anatomy. 3rd edition. By Theodore Shennan. Baltimore: William Wood & Company, pp. viii + 716, 1935. $9.00.

The principal revisions in this edition are those dealing with endocarditis, tuberculosis of the lungs, gastric ulcers, the splenomegalies, nephritis, and nephropathies. The general arrangement of the book follows the usual order of procedures in performing a necropsy and the description of lesions is limited to gross appearance. Some notable omissions from the text are those which would deal with methods of obtaining permission to perform a necropsy, the examination of bodies of those suffering violent death, in particular, traumatic accidents, the identification of bodies and parts of bodies, the methods of procuring material for bacteriological study and the application of bacteriology to the necropsy. The technic given follows the usual methods but the author's method of tying the intestine on itself is certainly not as good as the use of a ligature. The medicolegal section is written from the British viewpoint and the small section on animal parasites has inexcusable errors in nomenclature.

The book will be of primary use to medical students and internes majoring in pathology.

The Patient and the Weather. Vol. 1, part 1. By William F. Petersen. Ann Arbor: Edwards Brothers, Inc., pp. xx + 127, 1935. $3.75.

This is the introductory volume to a series, some of which have appeared, dealing with the effect of the weather on the patient. It presents the general thesis that most, if not all, of man's ailments of the mind and body are the result of the cyclonic disturbances of the earth, at least in the United States. The

evidence is fortified by what on first appearance seems to be statistical proof and by numerous spot maps. The writings of Hippocrates and the philosophy of Asclepius are used freely to support the premise. Careful examination, however, discloses no evidence of the concept of probable error and leaves the reader wondering just how reliable the figures are or if the author has considered other factors save the weather. As collateral reading, it is suggested that Jastrow's recent book, "Wish and Wisdom" might be read with profit both to the author and the reader.

Lobar Pneumonia and Serum Therapy. By Frederick T. Lord and Roderick Heffron. New York: The Commonwealth Fund, pp. 91, 1936. $1.00.

This little monograph contains the results of a state-wide (Massachusetts) study of pneumonia and its treatment. The authors review over 900 patients with lobar pneumonia who were treated with serum by 400 physicians. The six most common types of pneumococci are responsible for 84.1 per cent of cases of lobar pneumonia and Type I is responsible for 33.4 per cent. Lobar pneumonia comprises more than half of the fatal cases of all types of pneumonia and is seventh among the most common causes of death in the United States. The authors decide that the use of serum by general practitioners is entirely feasible and when administered within 96 hours of the onset of the disease should be expected to reduce the death rate by approximately half when the pneumonia is due to Types I and II.

Synopsis of Clinical Laboratory Methods. By W. E. Bray. St. Louis: The C. V. Mosby Co., pp. 324, 1936. $3.75.

This manual is the outgrowth of years of teaching and experience in the clinical laboratory and is an effort to bring together for ready reference the most frequently used laboratory methods. The introductory chapter gives some general rules for laboratory work, then follow chapters on urinalysis, hematology, blood chemistry, gastric analysis, feces, and intestinal parasites, body fluid examinations, sputum, bacteriology, water and milk

examinations, serology, basal metabolism, allergy tests, poisons, surgical pathology and indicators, stains et cetera.

The methods are given briefly and clearly without entering into the theory or explanation of the test. The manual will be useful as a laboratory guide to be used on the laboratory table.

Delafield and Prudden's Text-book of Pathology. 16th Ed. REVISED BY F. C. WOOD. Baltimore: William Wood & Company, pp. vi + 1339, 1936. $10.00.

This wellknown and much used text is now issued in its golden jubilee edition, the second important text on pathology issued in America. The attitude of the editor is clearly expressed in his preface in these words: "—modernity is largely only a changing of the labels on the specimen bottles. People still have the same lesions that they had when Delafield and Prudden wrote the first edition." Surely Dr. Wood does not think pathology has made no advances in a half century other than in the matter of labeling! True to his stated belief, one finds little changed in the text except for an essentially new chapter on the nervous system by E. M. Deery and in it most of the seventy new illustrations are found. Perhaps there were too many bottles to relabel in this field! There has been some revision of the chapters on muscles, bones and joints. The chapters on bacteriology and parasitology are long out of date and one may seriously question the propriety of retaining them in a general text of this nature. The result of many revisions gives rise to curious expressions for the year 1936 as for example: "More recently Theobald Smith, et cetera." This reference refers to work published thirty years ago.

In the field of general and special histologic pathology, the text is still authoritative and will undoubtedly remain so in spite of discoveries other than that bottles are poorly labeled.